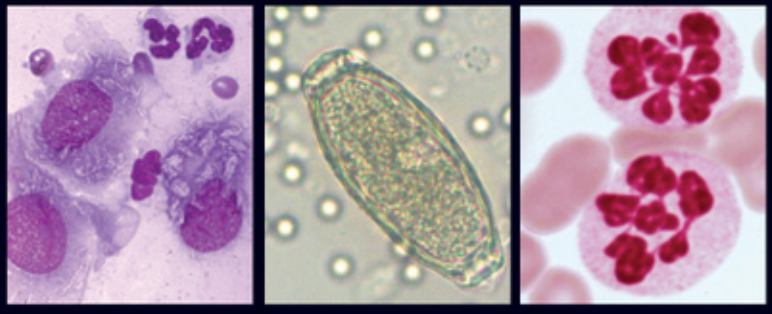
Duncan & Prasse's Veterinary Laboratory Medicine

CLINICAL PATHOLOGY

FIFTH EDITION

Kenneth S. Latimer



WILEY-BLACKWELL

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

DUNCAN & PRASSE'S VETERINARY LABORATORY MEDICINE: CLINICAL PATHOLOGY

Fifth Edition

www.Ebook777.com

Companion website

This book is accompanied by a companion website:

www.wiley.com/go/latimer

The website includes:

- All images from the book for downloading in PowerPoint
- All references hyperlinked to PubMed

Free ebooks ==> www.Ebook777.com

DUNCAN & PRASSE'S VETERINARY LABORATORY MEDICINE: CLINICAL PATHOLOGY

Fifth Edition

KENNETH S. LATIMER

WILEY-BLACKWELL
A John Wiley & Sons, Inc., Publication

www.Ebook777.com

This edition first published 2011 © 2011 by John Wiley & Sons, Inc.
Previous editions © 1977, 1986, 1994, 2003 Iowa State University Press

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 2121 State Avenue, Ames, Iowa 50014-8300, USA
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Blackwell Publishing, provided that the base fee is paid directly to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license by CCC, a separate system of payments has been arranged. The fee codes for users of the Transactional Reporting Service are ISBN-13: 978-0-8138-2014-9/2011.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloging-in-Publication Data
Duncan & Prasse's veterinary laboratory medicine : clinical pathology / [edited by] Kenneth S. Latimer. – 5th ed.
p. ; cm.
Duncan and Prasse's veterinary laboratory medicine
Veterinary laboratory medicine
Rev. ed. of: Duncan & Prasse's veterinary laboratory medicine / Kenneth S. Latimer, Edward a. Mahaffey, Keith W. Prasse. 4th ed. 2003.
Includes bibliographical references and index.
ISBN 978-0-8138-2014-9 (hardcover : alk. paper) 1. Veterinary clinical pathology. I. Latimer, Kenneth S. II. Duncan, J. Robert. III. Latimer, Kenneth S. Duncan & Prasse's veterinary laboratory medicine. IV. Title: Duncan and Prasse's veterinary laboratory medicine. V. Title: Veterinary laboratory medicine.
[DNLM: 1. Pathology, Veterinary–Outlines. 2. Pathology, Clinical–Outlines. SF 772.6]
SF772.6.D86 2011
636.089'607–dc22
2010049553

A catalogue record for this book is available from the British Library.

Set in 9.5/11.5 pt Berkeley by Toppan Best-set Premedia Limited

Disclaimer
The publisher and the author make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation warranties of fitness for a particular purpose. No warranty may be created or extended by sales or promotional materials. The advice and strategies contained herein may not be suitable for every situation. This work is sold with the understanding that the publisher is not engaged in rendering legal, accounting, or other professional services. If professional assistance is required, the services of a competent professional person should be sought. Neither the publisher nor the author shall be liable for damages arising herefrom. The fact that an organization or Website is referred to in this work as a citation and/or a potential source of further information does not mean that the author or the publisher endorses the information the organization or Website may provide or recommendations it may make. Further, readers should be aware that Internet Websites listed in this work may have changed or disappeared between when this work was written and when it is read.

1 2011

Free ebooks ==> www.Ebook777.com

TABLE OF CONTENTS

CONTRIBUTING AUTHORS vii

PREFACE ix

ACKNOWLEDGMENTS xi

CHAPTER 1. ERYTHROCYTES 3

CHAPTER 2. LEUKOCYTES 45

CHAPTER 3. HEMATOPOIETIC NEOPLASIA 83

CHAPTER 4. HEMOSTASIS 107

CHAPTER 5. WATER, ELECTROLYTES, AND ACID BASE 145

CHAPTER 6. PROTEINS, LIPIDS, AND CARBOHYDRATES 173

CHAPTER 7. LIVER 211

CHAPTER 8. DIGESTIVE SYSTEM 231

CHAPTER 9. URINARY SYSTEM 253

CHAPTER 10. MUSCLE 283

CHAPTER 11. ENDOCRINE SYSTEM 295

CHAPTER 12. CYTOLOGY 331

CHAPTER 13. GENERATING AND INTERPRETING TEST RESULTS: TEST VALIDITY, QUALITY CONTROL, REFERENCE VALUES, AND BASIC EPIDEMIOLOGY 365

CASE STUDIES 383

INDEX 475

Companion website

This book is accompanied by a companion website:
www.wiley.com/go/latimer

The website includes:

- All images from the book for downloading in PowerPoint
- All references hyperlinked to PubMed

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CONTRIBUTING AUTHORS

Perry J. Bain, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Assistant Professor
Department of Biomedical Sciences
Large Animal Hospital
Cummings School of Veterinary Medicine at Tufts University
North Grafton, MA 01536

Holly S. Bender, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Professor
Department of Veterinary Pathology
College of Veterinary Medicine
Iowa State University
Ames, IA 50011

Dorothee Bienzle, DVM, MSc, PhD
Diplomate, American College of Veterinary Pathologists
Professor and Canada Research Chair in Veterinary Pathology
Department of Pathobiology
Ontario Veterinary College
University of Guelph
Guelph, Ontario, Canada N1G 2W1

Mary K. Boudreaux, DVM, PhD
Professor
Department of Pathobiology
College of Veterinary Medicine
Auburn University, AL 36849

Denise I. Bounous, DVM, PhD
Medical Technologist (American Society of Clinical Pathologists)
Diplomate, American College of Veterinary Pathologists
Group Director, Drug Safety Evaluation
Bristol-Myers Squibb
Princeton, NJ 08543

Charles W. Brockus, DVM, PhD
Diplomate, American College of Veterinary Internal Medicine
Diplomate, American College of Veterinary Pathologists
Charles River
Reno, NV 89511

Ellen W. Evans, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Senior Director, Immunotoxicology Center of Emphasis
Pfizer, Inc.
Groton, CT 06340

Duncan C. Ferguson, VMD, PhD
Diplomate, American College of Veterinary Internal Medicine
Diplomate, American College of Veterinary Clinical Pharmacology
Professor and Head
Department of Veterinary Biosciences
College of Veterinary Medicine
University of Illinois at Urbana-Champaign
Urbana, IL 61802

Jeanne W. George, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Professor Emeritus
Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine
University of California-Davis
Davis, CA 95616

www.Ebook777.com

Christopher R. Gregory, DVM, PhD
Medical Technologist (American Society of Clinical Pathologists)
Associate Research Scientist
Department of Small Animal Medicine
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602

Robert L. Hall, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Covance Laboratories, Inc.
Madison, WI 53704

Margarethe Hoenig, Dr med vet, PhD
Professor
Department of Veterinary Clinical Medicine
College of Veterinary Medicine
University of Illinois at Urbana-Champaign
Urbana, IL 61802

Paula M. Krimer, DVM, DVSc
Diplomate, American College of Veterinary Pathologists
Assistant Professor
Athens Veterinary Diagnostic Laboratory
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602

Kenneth S. Latimer, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Covance Laboratories, Inc.
Vienna, VA 22182
and
Professor Emeritus
Department of Pathology
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602

Pauline M. Rakich, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Professor
Athens Veterinary Diagnostic Laboratory
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602

Elizabeth A. Spangler, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Diplomate, American College of Veterinary Internal Medicine
Assistant Professor
Department of Pathobiology
College of Veterinary Medicine
Auburn University, AL 36849

Heather L. Tarpley, DVM
Diplomate, American College of Veterinary Pathologists
Chestatee Animal Hospital
Dahlonega, GA 30533

Niraj K. Tripathi, BVScAH, MVSc, PhD
Diplomate, American College of Veterinary Pathologists
Covance Laboratories, Inc.
Madison, WI 53704

Julie L. Webb, DVM
Diplomate, American College of Veterinary Pathologists
Instructor
Department of Pathobiological Sciences
College of Veterinary Medicine
University of Wisconsin
Madison, WI 53706

Elizabeth G. Welles, DVM, PhD
Diplomate, American College of Veterinary Pathologists
Professor
Department of Pathobiology
College of Veterinary Medicine
Auburn University
Auburn, AL 36849

Shanon M. Zabolotzky, DVM
Diplomate, American College of Veterinary Pathologists
Clinical Pathologist
IDEXX Laboratories, Inc.
West Sacramento, CA 95605

Free ebooks ==> www.Ebook777.com

PREFACE

The publication of the fifth edition of *Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology* represents a collation and distillation of educational information about veterinary clinical pathology that spans almost 40 years. Prior to retirement, Drs. Duncan and Prasse (Figure 1) established a legacy of excellence in teaching, diagnostic service, and applied research in veterinary clinical pathology that endures to the present day.

This textbook has evolved along with the specialty of veterinary clinical pathology. Since the initial American College of Veterinary Pathologists (ACVP) certification of Drs. Duncan and Prasse in clinical pathology, some of the new authors of this text now represent the third generation of veterinary clinical pathologists. General authorship also has continued to expand and the expertise of all coauthors has added a new dimension and considerable depth to the fifth edition of this textbook. Hopefully, a new editor will continue this legacy in the future to educate another generation of veterinary students, interns, residents, clinicians, and practitioners in the science, art, and practice of interpreting laboratory data.



Dr. J. Robert Duncan, Professor Emeritus (left), and Dr. Keith W. Prasse, Retired Dean (right), The University of Georgia College of Veterinary Medicine.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

ACKNOWLEDGMENTS

This fifth edition of edition of *Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology* would not have been possible without the assistance of several new and many repeat authors. Their professional contributions to this textbook are gratefully acknowledged and will have a positive impact on patient diagnostics and care. I would also like to recognize colleagues, clinical and anatomic pathology residents, veterinary students, and practicing veterinarians who have continued to provide the professional feedback that has improved the revision of this textbook. Kip Carter, MS, CMI, at The University of Georgia College of Veterinary Medicine again revised several line drawings. Erica Judisch, Nancy Turner, and the production staff at Wiley-Blackwell provided the professional assistance that was essential for the timely printing of this new edition. With the completion of this project, I can once again enjoy life with the whippets at home, in the show ring, and on the lure coursing field.



Cody (FC Longlesson Private Conversation) on the field and looking for action. Image courtesy of Julie Poole Photography, Knoxville, TN.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

DUNCAN & PRASSE'S VETERINARY LABORATORY MEDICINE: CLINICAL PATHOLOGY

Fifth Edition

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 1

ERYTHROCYTES

Charles W. Brockus, DVM, PhD

BASIC CONCEPTS OF ERYTHROCYTE FUNCTION, METABOLISM, PRODUCTION, AND BREAKDOWN

I. THE ERYTHRON

A. This widely dispersed mass of erythroid cells includes circulating erythrocytes and bone marrow precursor, progenitor, and stem cells.

B. Its function is oxygen transport, which is mediated by hemoglobin.

C. Hemoglobin is transported in erythrocytes whose membrane, shape, cytoskeleton, and metabolic processes ensure survival of the cell against the stresses of circulation and various injurious substances.

D. Hemoglobin consists of heme and globin, and each complete hemoglobin molecule is a tetramer.

1. Each heme moiety contains an iron atom in the 2^+ valence state (Fe^{2+}).

2. A globin chain of specific amino acid sequence is attached to each heme group.

3. The complete hemoglobin molecule is a tetramer, containing four heme units and four globin chains. The globin chains are identical pairs (dimers), designated as α-chains or δ-chains.

II. HEME SYNTHESIS

A. Heme synthesis is unidirectional and irreversible. It is controlled at the first step by the enzyme δ-aminolevulinic acid synthase, whose synthesis is controlled by negative feedback from heme concentration within the erythrocyte.

1. Lead inhibits most of the steps in heme synthesis to some degree. Lead also inhibits the delivery of iron to the site of ferrochelatase activity.

2. Chloramphenicol may inhibit heme synthesis.

B. Porphyrins and their precursors are the intermediates of heme biosynthesis.

1. Certain enzyme deficiencies in the synthetic pathway can lead to excessive accumulation of porphyrins and their precursors.

2. These excesses of porphyrins and their precursors are called porphyrias.

3. Porphyrias vary in the intermediate products that accumulate and in their clinical manifestations.

4. These excess porphyrins escape the erythrocyte and may be deposited in the tissues or excreted in the urine and other body fluids.

C. After formation of protoporphyrin, iron is inserted into the molecule by ferrochelatase, and heme is formed.

www.Ebook777.com

III. GLOBIN SYNTHESIS

A. Each hemoglobin molecule is comprised of four globin chains, each of which binds to a heme group.
- **1.** The hemoglobin type depends on the type of globin chains, which are determined by amino acid sequences.
  - **a.** Embryonic, fetal, and adult hemoglobins are found in various animals.
  - **b.** The presence and number of each hemoglobin type vary with the species.
- **2.** Heme and globin synthesis are balanced (increase in one results in an increase in the other).

B. Abnormalities in globin synthesis (i.e., hemoglobinopathies) have not been described in domestic animals.

IV. IRON METABOLISM

Body iron metabolism/content is based on an extremely efficient system of conservation and recycling that is regulated by the rate of duodenal absorption rather than excretion. Hepcidin is a recently identified 25 amino acid peptide (bioactive form) produced within the liver and transported within the blood by α-2-macroglobulin. It has been found to play a key role in mediation of iron metabolism. In short, increased hepcidin is accompanied by a decrease in iron availability, whereas decreased Hepcidin is associated with an increase in iron availability. Hepcidin is a component of the type II acute phase response induced by interleukin-6 and controls plasma iron concentration by inhibiting iron export by ferroportin from enterocytes and macrophages. Absorption is regulated by the amount of storage iron (large iron stores decrease absorption) and rate of erythropoiesis (accelerated erythropoiesis increases absorption). Less than 0.05% of the total body iron is acquired or lost each day.

A. Iron is transported in blood bound to the δ-globulin, transferrin.
- **1.** Iron bound to transferrin is measured as serum iron (SI). This is an unreliable measure of total body iron stores.
  - **a.** Conditions with decreased SI
    - **(1)** Iron deficiency
    - **(2)** Acute and chronic inflammation or disease (including anemia of inflammatory disease)
    - **(3)** Hypoproteinemia
    - **(4)** Hypothyroidism
    - **(5)** Renal disease
  - **b.** Conditions with increased SI
    - **(1)** Hemolytic anemia
    - **(2)** Accidental lysis of erythrocytes during sampling (hemolysis)
    - **(3)** Glucocorticoid excess in the dog and horse. In contrast, SI is decreased in cattle with glucocorticoid excess.
    - **(4)** Iron overload, which may be an acquired (e.g., iron toxicity) or hereditary (e.g., hemochromatosis in Salers cattle) condition. Iron overload in some birds (e.g., mynahs and toucans) also may be hereditary.
    - **(5)** Nonregenerative anemia
  - **c.** SI can be expressed as a percentage of total iron-binding capacity (TIBC, see below) and reported as the percent saturation.
- **2.** TIBC is an indirect measurement of the amount of iron that transferrin will bind. An immunologic method is available to quantitate transferrin, but is not used commonly.
  - **a.** Only one-third of transferrin binding sites usually are occupied by iron. This is expressed as percent saturation.
  - **b.** TIBC is increased in iron deficiency in most species except the dog.

Free ebooks ==> www.Ebook777.com

3. Transferrin can bind more iron than is normally present. Therefore, the numeric difference between TIBC and SI is the amount of iron-binding capacity remaining on transferrin or the unbound iron-binding capacity (UIBC).

B. Hepcidin has been found to be the main regulator of iron homeostasis; it is produced in the liver and acts systemically in iron overloading (increased) or in response to anemia or hypoxia (decreased). Hephaestin (an intestinal ceruloplasmin analog) and ceruloplasmin (synthesized in the liver) are both copper-containing proteins involved in iron transport. Ceruloplasmin also is an acute phase inflammatory reactant. Ferroportin 1 and divalent metal transporter 1 (DMT1) are necessary for transfer of iron from intestinal epithelium and macrophages to serum transferrin. Hepcidin induces the internalization and degradation of ferroportin, thereby inhibiting iron transport.

C. Iron is incorporated into hemoglobin during the last step of heme synthesis. Lack of intracellular iron causes an increase in erythrocyte protoporphyrin concentration.

D. Iron is stored in macrophages as ferritin and hemosiderin.

E. Ferritin is a water-soluble iron-protein complex.

1. Ferritin is the more labile storage form of iron.

2. Small amounts circulate that can be measured as serum ferritin, which is an indirect measurement of the storage iron pool. A species-specific immunoassay is required.

a. Serum ferritin concentration is decreased in iron deficiency.

b. Serum ferritin concentration is increased in the following:

(1) Hemolytic anemia

(2) Iron overload

(3) Acute and chronic inflammation

(4) Liver disease

(5) Some neoplastic disorders (e.g., lymphoma, malignant histiocytosis)

(6) Malnutrition (cattle)

F. Hemosiderin is a more stable, but less available, storage form of iron that is comprised of native and denatured ferritin and protein. It is not water-soluble and is stainable within tissues by Perl's or Prussian blue techniques.

G. Abnormalities in serum iron are related to absorptive failures, nutritional deficiencies, iron loss via hemorrhage, and aberrant iron metabolism with diversion to macrophages at the expense of hematopoietic cells (which occurs in chronic disease processes and inflammation).

V. ERYTHROCYTE METABOLISM

Metabolism is limited after the reticulocyte stage because mature erythrocytes lack mitochondria for oxidative metabolism. Biochemical pathways found in mature erythrocytes are listed in Figure 1.1 with their functions and associated abnormalities.

A. Embden-Meyerhof pathway

1. By this anaerobic pathway, glycolysis generates adenosine triphosphate (ATP) and NADH. ATP is essential for membrane function and integrity, whereas NADH is used to reduce methemoglobin.

2. Important enzymes in this pathway include pyruvate kinase (PK) and phosphofructokinase (PFK). Enzyme deficiencies in this pathway can lead to hemolytic anemia (e.g., PK and PFK deficiency anemias of dogs).

3. PK deficiency impairs ATP production, resulting in a macrocytic hypochromic anemia with 15% to 50% reticulocytes, myelofibrosis, hemochromatosis, decreased erythrocyte lifespan, and accumulation of phosphoenolpyruvate (PEP) and 2,3 diphosphoglyceric acid (DPG). PK deficiency has been reported in dogs (Basenji, West Highland White Terrier, Cairn Terrier, American Eskimo Dog, Miniature Poodle, Pug, Chihuahua, and Beagle) and cats (Abyssinian and Somali).

www.Ebook777.com

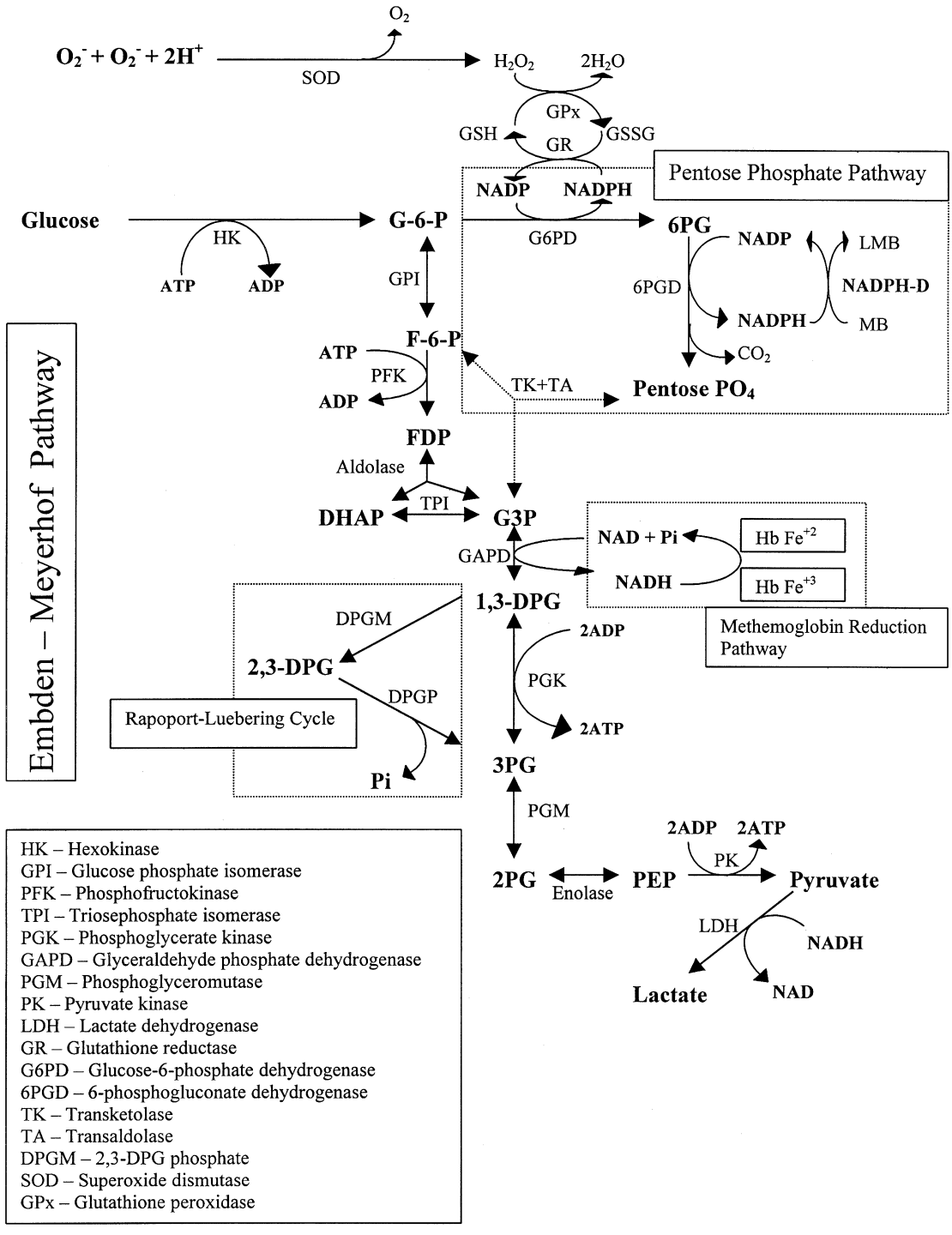


FIGURE 1.1. A schematic diagram showing the major erythrocyte metabolic pathways that provide energy and protect from oxidative injury.

Free ebooks ==> www.Ebook777.com

4. PFK deficiency results in decreased erythrocytic 2,3 DPG concentration, hematocrit (Hct) that is within the reference interval or decreased, persistent reticulocytosis, and alkalemia leading to hemolysis. This enzyme deficiency is reported in dogs (English Springer Spaniels, Cocker Spaniels, and some mixed-breed dogs).

B. Pentose phosphate pathway (Hexose-monophosphate pathway)

1. Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme in this anaerobic pathway.

2. This pathway produces NADPH, which is a major reducing agent in the erythrocyte. NADPH serves as a co-factor for the reduction of oxidized glutathione. Reduced glutathione neutralizes oxidants that can denature hemoglobin.

3. A deficiency or defect in glucose-6-phosphate dehydrogenase results in hemolytic anemia under conditions of mild oxidative stress (e.g., glucose-6-phosphate dehydrogenase deficiency in the horse with eccentrocytes and Heinz bodies).

C. Methemoglobin reductase pathway

1. Hemoglobin is maintained in the reduced state (i.e., oxyhemoglobin; Fe^{2+}) necessary for transport of oxygen by this pathway.

2. Enzyme deficiency results in methemoglobin accumulation. Methemoglobin (Fe^{3+}) cannot transport oxygen, and cyanosis results. With substantially increased methemoglobin concentration, the blood and mucous membranes may appear brown.

3. NADH and NADPH methemoglobin reductases also are present. The former predominates in normal conditions and the latter is activated by redox dyes (e.g., methylene blue).

4. Methemoglobin reductase deficiency results in cyanosis, methemoglobinemia, pO_2 within the reference interval, and exercise intolerance. This deficiency has been reported in dogs (American Eskimo Dog, Poodle, Cocker Spaniel-Poodle cross, Chihuahua, and Borzoi).

D. Rapoport-Luebering pathway

1. This pathway allows formation of 2,3 diphosphoglycerate (2,3 DPG), which has a regulatory role in oxygen transport. Increased 2,3 DPG favors oxygen release to tissues by lowering the oxygen affinity of hemoglobin.

2. Depending upon the species, some anemic animals usually have increased 2,3 DPG concentrations and deliver more oxygen to tissues with a lesser amount of hemoglobin (a compensatory mechanism).

3. Animal erythrocytes vary in the concentration of 2,3 DPG and its reactivity with hemoglobin. Dog, horse, and pig erythrocytes have high concentrations and reactivity, whereas cat and ruminant erythrocytes have low concentrations and reactivity.

VI. ERYTHROKINETICS

A. Stem cells, progenitor cells, and precursor cells (Figure 1.2)

1. Pluripotential and multipotential stem cells (CFU-GEMM or CD34+ cells)

a. These cells have the capacity for self-renewal and differentiate into progenitor cells.

b. Differentiation is controlled by growth-promoting stimuli produced by marrow stromal cells. A variety of growth factors and cytokines are involved (SCF, IL-3, IL-9, IL-11, and erythropoietin).

c. When a stem cell differentiates, it loses some of its ability to self-replicate and also loses some of its potentiality.

2. Progenitor cells

a. Some early progenitor cells have the capability of differentiating into more than one cell line (e.g., CFU-GEMM has the potential to differentiate into granulocytes, erythrocytes, monocytes, or megakaryocytes).

www.Ebook777.com

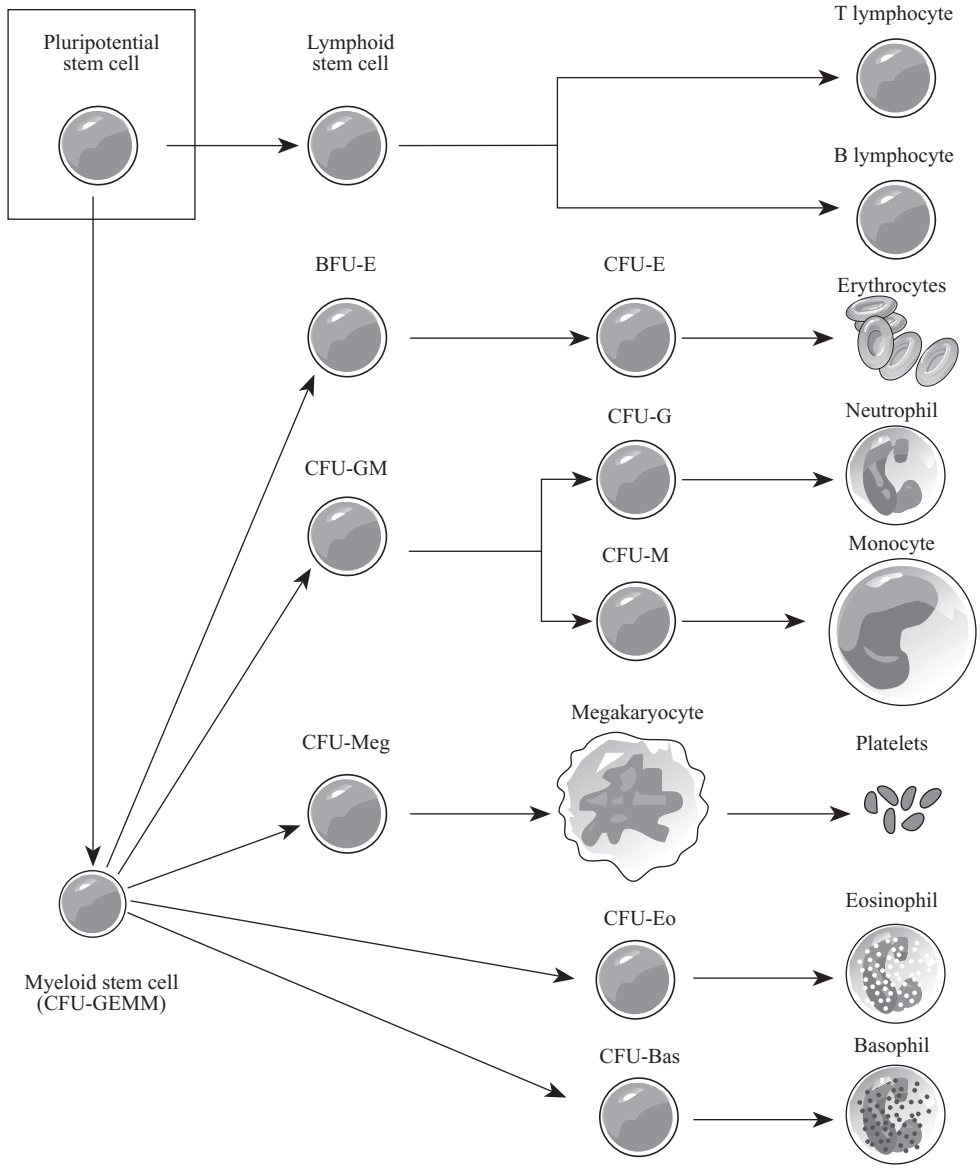


FIGURE 1.2. A model of hematopoiesis. The pluripotential stem cell gives rise to lymphoid and myeloid multipotential stem cells. The lymphoid stem cell differentiates into T- and B-lymphocytes. The myeloid stem cell (CFU-GEMM) forms progenitor cells that include erythroid burst-forming units (BFU-E), which differentiate into erythroid colony-forming units (CFU-E); granulocyte/monocyte colony-forming units (CFU-GM), which differentiate into granulocyte colony-forming units (CFU-G) and monocyte colony-forming units (CFU-M); megakaryocytic colony-forming units (CFU-Meg); eosinophil colony-forming units (CFU-Eo); and basophil colony-forming units (CFU-Bas). These colony-forming units differentiate into precursor cells, then mature cells, of the various cell lines.

Free ebooks ==> www.Ebook777.com

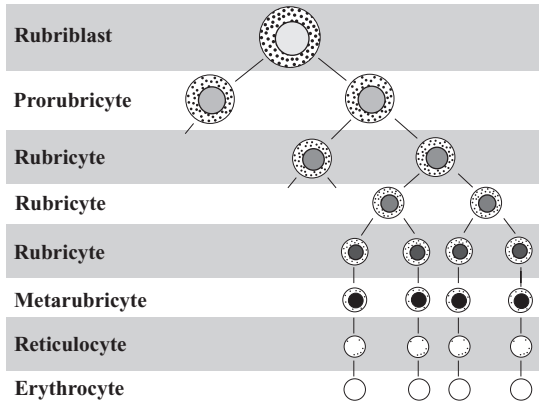


FIGURE 1.3. Sequence of erythropoiesis.

b. Other progenitor cells are unipotential (e.g., CFU-E can only differentiate into erythroid cells).

c. Progenitor cells have limited capacity for self renewal and differentiate into precursor cells of the various cell lines.

d. Progenitor cells are not recognizable morphologically with Romanowsky stains, but resemble small lymphocytes.

3. Precursor cells

a. Precursor cells have no capacity for self-renewal but proliferate while differentiating into the mature, functional cells.

b. These are the first cells that can be recognized as members of a particular cell line.

B. Erythropoiesis (Figure 1.3)

1. In mammals, erythropoiesis occurs extravascularly in bone marrow parenchyma. In avian species, erythropoiesis occurs within the vascular sinuses of the bone marrow (intravascular or intrasinusoidal development).

2. Characteristic morphologic changes take place during maturation from the rubriblast to the mature erythrocyte (Figure 1.4).

a. Cells become smaller.

b. Nuclei become smaller and their chromatin is more aggregated:

(1) Cell division stops in the late rubricyte stage when a critical intracellular concentration of hemoglobin is reached.

(2) The nucleus is extruded at the metarubricyte state, and a reticulocyte is formed in mammals. In contrast, avian reticulocytes and mature erythrocytes retain their nuclei.

c. Cytoplasmic color changes from blue to orange as hemoglobin is formed and RNA is lost.

3. In mammals, reticulocytes and erythrocytes migrate into the venous sinus of the bone marrow through transient apertures in endothelial cell cytoplasm.

a. Reticulocytes of most species remain in the bone marrow for two to three days before release and ultimately mature in the peripheral blood or spleen.

b. In health, the reticulocytes of cattle and horses mature in the bone marrow; mature erythrocytes are released.

4. The time from stimulation of the erythropoietic progenitor cell until reticulocytes are released is approximately five days.

5. Starting with the rubriblast, three to five divisions produce eight to 32 differentiated cells.

6. The bone marrow has the capacity to increase erythropoiesis.

www.Ebook777.com

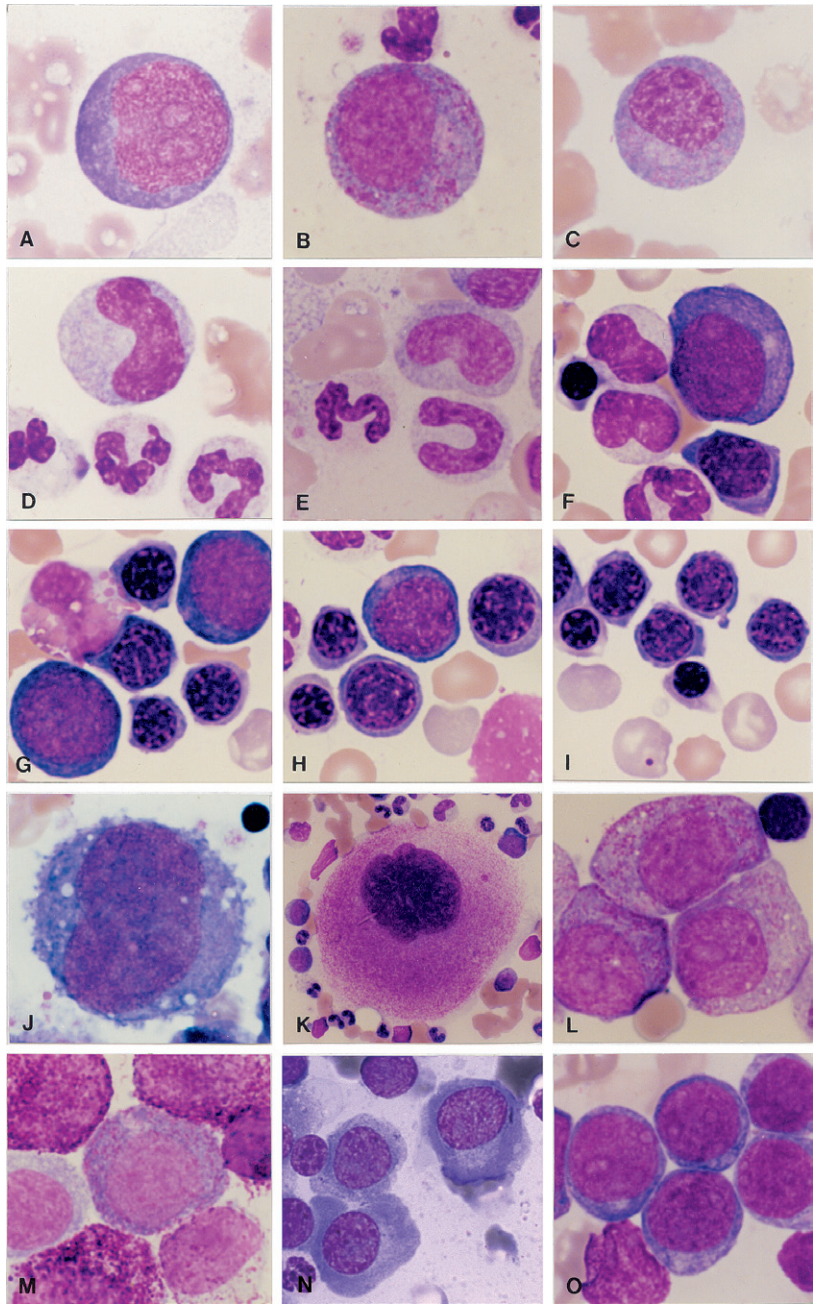


FIGURE 1.4. Normal hematopoietic cells and leukemic cells in bone marrow. A. myeloblast; B. promyelocyte; C. neutrophil myelocyte; D. neutrophil metamyelocyte and segmenters; E. neutrophil metamyelocyte, band, and segmenter; F. rubriblast, rubricyte, metarubricyte, two neutrophil metamyelocytes, and a neutrophil segmenter; G. two prorubricytes, four rubricytes, and an eosinophil; H. five rubricytes; I. five rubricytes, a metarubricyte, and a polychromatophilic erythrocyte with a Howell-Jolly body; J. immature megakaryocyte with blue, granular cytoplasm; K. mature megakaryocyte with granular, pink cytoplasm (low magnification); L. promyelocytes in canine myeloblastic leukemia; M. poorly differentitied mast cells in feline mast cell leukemia; N. plasma cells in canine plasma cell myeloma; O. lymphoblasts in canine acute lymphocytic leukemia (Wright-Leishman stain).

Free ebooks ==> www.Ebook777.com

a. Erythrocyte production can be increased up to seven times the normal rate in humans, providing the necessary stimulation and nutrients are present. This capacity to increase production varies with the animal species. It is greatest in birds and dogs and least in cattle and horses.

b. An increase in the number of erythrocytes delivered to the blood occurs primarily via increased stem cell input and, to a lesser extent, by a shortened maturation time.

c. Erythrocytes may be delivered to the circulation faster by earlier reticulocyte release and skipped cell divisions. These processes do not increase the total number of erythrocytes produced and are of temporary benefit.

7. Regulation of erythropoiesis

a. Erythropoietin (Epo)

(1) The majority of Epo is produced by peritubular interstitial cells of the kidney in response to hypoxia, but the liver may account for 10% to 15% of Epo production by specific hepatocytes and Ito cells.

(2) Actions of Epo

(a) Inhibition of apoptosis of newly formed progenitor cells and prorubricytes, allowing them to differentiate into mature erythrocytes.

(b) Stimulation of hemoglobin synthesis in already dividing erythroid cells.

(c) Switching of hemoglobin synthesis in sheep from one adult type to another (i.e., HbA to HbC).

b. Interleukin-3 (IL-3) and colony-stimulating factors (GM-CSF and G-CSF).

(1) IL-3 is produced by activated T-lymphocytes; GM-CSF by activated T-lymphocytes, macrophages, endothelial cells, and fibroblasts; and G-CSF by macrophages, monocytes, neutrophils, endothelial cells, and fibroblasts.

(2) In concert with Epo, these factors stimulate the multiplication of a primitive erythroid progenitor cell, BFU-E, and its differentiation into the CFU-E progenitor cell.

(3) The BFU-E progenitor cell is rather insensitive to Epo stimulation alone.

c. Androgens increase Epo release. In contrast, estrogens and corticosteroids decrease Epo release, but their effect is probably not clinically significant.

d. Thyroid and pituitary hormones alter the tissue demands for oxygen, thereby changing the requirement for erythropoiesis.

VII. ERYTHROCYTE DESTRUCTION

A. The average erythrocyte lifespan in circulation varies with the species: cow, 160 days; sheep, 150 days; horse, 145 days; dog, 110 days; pig, 86 days; cat, 70 days; bird, approximately 35 days. Thus, ruminant blood smears have infrequent reticulocytosis in health, while avian blood smears may have 4% to 5% reticulocytes in health. In certain disease states, anemia may develop more quickly in birds and cats than in large animals because of the normally short erythrocyte lifespan.

B. Aging of erythrocytes is accompanied by changes in enzyme content and cell membrane structure that make the cells less capable of survival and subject to removal by the spleen.

C. In health, senescent erythrocytes are removed from circulation by two routes.

1. Phagocytosis by macrophages is the major route of senescent erythrocyte removal (Figure 1.5).

a. Within the phagosome, the erythrocyte releases its hemoglobin, which is split into heme and globin.

b. Globin is broken down to its constituent amino acids, which are reutilized.

c. After releasing the iron, heme is cleaved by heme oxygenase, forming carbon monoxide and biliverdin.

www.Ebook777.com

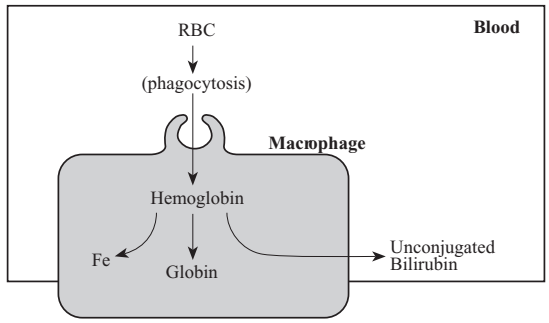


FIGURE 1.5. Pathway of phagocytic (extravascular) destruction of erythrocytes.

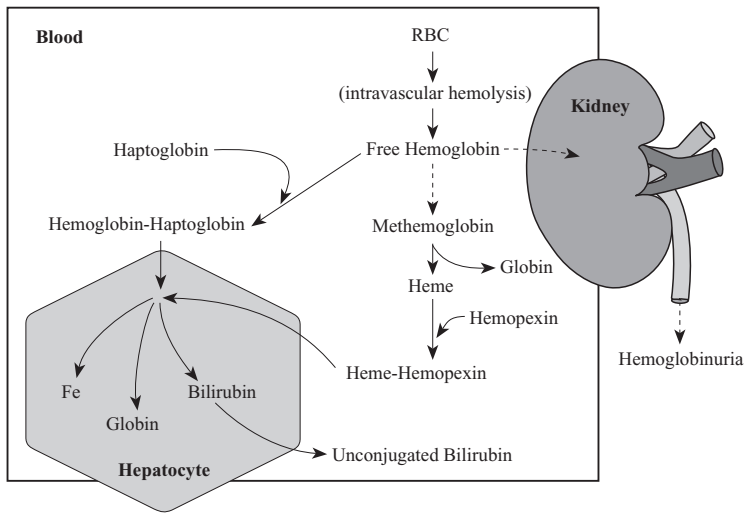


FIGURE 1.6. Pathway of intravascular destruction of erythrocytes.

d. Biliverdin is reduced by biliverdin reductase to bilirubin, which is excreted into the blood, where it binds with albumin for transport to the liver. Birds lack biliverdin reductase; therefore, they form biliverdin as an end product and not bilirubin. Biliverdin is green, which gives the characteristic color to bruises in avian tissue.

2. Intravascular lysis with release of hemoglobin into plasma is a minor route of senescent erythrocyte removal (Figure 1.6).

a. Free hemoglobin in the plasma binds to the α_2-globulin, haptoglobin. The hemoglobin-haptoglobin complex is cleared from plasma by the liver, preventing loss of hemoglobin in the urine. Enough haptoglobin usually is present to bind 150 mg/dL of hemoglobin. Plasma appears pink to red when 50 to 100 mg/dL of hemoglobin is present; therefore, discoloration of plasma precedes hemoglobinuria. In health, plasma discoloration is not observed.

b. If intravascular lysis is excessive, the serum haptoglobin may become saturated. The free hemoglobin then dissociates into dimers, which can pass the glomerular filter. This does not occur in health.

c. With time, free hemoglobin in the plasma is oxidized to methemoglobin, which dissociates to free ferriheme, which complexes with the β-globulin, hemopexin.

Free ebooks ==> www.Ebook777.com

d. The heme-hemopexin complexes are cleared by the liver, again preventing hemoglobin loss in the urine.

e. Hemoglobin that passes into the glomerular filtrate is absorbed by the proximal tubules and catabolized to iron, bilirubin, and globin.

f. Unabsorbed hemoglobin passes into the urine, causing hemoglobinuria.

g. Tubular epithelial cells containing hemosiderin may slough into the urine, producing hemosiderinuria.

3. Similar routes of destruction occur in hemolytic anemia, but either extravascular or intravascular hemolysis will predominate.

MEANS OF EVALUATING ERYTHROCYTES

I. HEMATOCRIT (HCT), HEMOGLOBIN (HB) CONCENTRATION, AND RED BLOOD CELL (RBC) COUNT ARE INDICATORS OF CIRCULATING RBC MASS. COMPUTER GRAPHICS OF AUTOMATED HEMATOLOGY ANALYZERS ARE SENSITIVE IN DETECTING CHANGES IN ERYTHROCYTE VOLUME OR HB CONCENTRATIONS.

A. Hct is the percent of blood comprised of erythrocytes.

1. Centrifugal methods give a packed-cell volume (PCV), a very accurate measurement with small inherent error (±1%).

a. Plasma obtained by this method can be used for other routine determinations.

(1) Plasma protein concentration using refractometry

(2) Plasma fibrinogen concentration using heat precipitation and refractometry

(3) Plasma color and transparency

(a) Normal plasma is clear and colorless (dog and cat) to light yellow (horse and cow).

(b) Icteric plasma is yellow and clear.

(c) Hemoglobinemic plasma is pink to red and clear.

(d) Lipemic plasma is whitish to pink and opaque.

b. The buffy coat zone, a white layer between the RBCs and plasma, is comprised of leukocytes and platelets. Measurement of its width has been used to estimate white blood cell counts.

c. Microfilaria may be detected by microscopic examination of the plasma just above the buffy coat layer.

2. Most automated cell counters, designed for human blood, calculate the Hct after determining the RBC count and the mean corpuscular volume of the erythrocyte population. The formula for this calculation is Hct % = (RBC/μL) × MCV (fL). The potential for error is greater than the PCV method, because only dogs have an erythrocyte volume that is comparable to human RBCs. Other domestic mammals have smaller erythrocytes than humans. However, newer and more advanced hematology analyzers can be modified easily to identify blood cells of many different species. Birds have elliptical, nucleated erythrocytes, which may interfere with automated RBC counts.

B. Hb concentration

1. Colorimetric determination by the cyanmethemoglobin technique or the newer cyanide-free hemoglobinhydroxylamine complex method is used most frequently. Avian erythrocytes must be lysed and the specimen must be centrifuged to remove free nuclei before Hb concentration can be accurately determined and indices calculated.

a. The coefficient of variation is approximately ±5%.

b. Heinz bodies, hemolysis, lipemia, and treatment with Oxyglobin® may cause false high values.

2. Some automated instruments directly measure optical density of oxyhemoglobin.

3. Hb concentration provides the most direct indication of oxygen transport capacity of the blood and should be approximately one-third the Hct if erythrocytes are of normal size.

www.Ebook777.com

4. Determination of Hb concentration provides no clinical advantage over the Hct other than allowing the calculation of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (see below). However, Hb concentration may be slightly more accurate in determining changes in circulating RBC mass when compared to Hct.
5. Newer hematology analyzers also generate a Hb concentration histogram.

C. RBC count
1. RBC counts, performed with a hemocytometer, have a large degree of error. Thus, hemacytometer-derived RBC counts are of limited value, except in avian species.
2. Automatic counters, if standardized for mammalian blood, allow for more accurate RBC counts. Automated counters are not well validated for avian blood, because all nucleated cells (RBCs, WBCs, and thrombocytes) are counted.
3. The primary value of the RBC count is that it allows determination of MCV and MCH (see below).

D. Factors affecting Hct, Hb concentration, and RBC count
1. Change in circulating RBC mass affects all three parameters.
   a. Low values occur in anemia. Decreases in the three parameters may be disproportionate if cell size and/or Hb content/cell also are altered.
   b. Increased RBC mass (absolute polycythemia) causes high values.
   c. Spuriously high values occur with dehydration and excitement-induced splenic contraction.
   d. Sighthound breeds such as the Greyhound, Saluki, Whippet, and Afghan hound normally have higher Hct/PCV values than other breeds, with values often found into the mid-60s.
2. Change in plasma volume affects all three parameters; therefore, interpretation must always be made with knowledge of the patient's hydration status (Cases 6, 9, 18, 24).
   a. Dehydration or fluid shifts to visceral organs cause increased values.
   b. Overhydration with parenteral fluids causes a reduction in values, simulating anemia.

II. RED BLOOD CELL INDICES. RBC INDICES ARE HELPFUL IN THE CLASSIFICATION OF CERTAIN ANEMIAS.

A. Mean corpuscular volume (MCV) represents the red cell volume in femtoliters (fL)
1. $(\text{PCV} \times 10) \div \text{RBC count (millions)} = \text{MCV (femtoliters)}$
2. The MCV is determined directly by automatic cell counters.
3. Factors affecting MCV values
   a. Reticulocytosis is the most common cause of macrocytosis (increased MCV) (Cases 1, 2). Reticulocytes, particularly early forms, are large cells.
   b. Immature animals of most species have small erythrocytes and microcytosis (low MCV). This also could reflect iron deficiency, which is more common in young animals.
   c. Iron deficiency causes microcytosis (Case 5). An extra cell division occurs before the critical cytoplasmic concentration of hemoglobin is reached that is necessary to stop DNA synthesis and cell division. Smaller cells subsequently are produced.
   d. Microcytosis occurs in dogs with portosystemic venous shunts (Case 13).
   e. Healthy Asian breeds of dogs (Akita, Chow Chow, Shar Pei, and Shiba Inu) often have microcytic erythrocytes.
   f. Greyhounds normally have a higher MCV than non-greyhounds that may be due to their erythrocyte's significantly shortened life span (approximately 55 days).
   g. Interference with nucleic acid synthesis causes an inhibition of cell division and thereby larger cells. Macrocytic anemia from an inherited, selective intestinal malabsorption of cobalamin (vitamin B_{12}) has been documented in Giant Schnauzers.
   h. Congenital macrocytosis occurs in Poodles.

Free ebooks ==> www.Ebook777.com

i. Hereditary stomatocytosis with macrocytosis has been observed in Alaskan Malamutes, Drentse-Partrijshond, and Miniature Schnauzers.
j. FeLV-infected cats often have macrocytic erythrocytes, possibly due to asynchronous maturation.
k. Erythrocyte agglutination can cause a false increase in the MCV.

B. Mean corpuscular hemoglobin (MCH) represents how much Hb is present within an average erythrocyte in picograms (pg)

1. (Hb concentration × 10) ÷ RBC count (millions) = MCH (picograms)
2. Factors affect the MCH and MCHC in a similar way (below); therefore, the MCH offers little additional hematologic information concerning the patient.
3. MCH is influenced by MCV. For example, smaller erythrocytes contain less Hb; therefore, they have a decreased MCH.
4. In some cases of iron deficiency anemia, MCH may decrease before MCHC decreases.
5. This index is not generally used in the classification of anemias. If the MCHC and MCH differ, interpretation of the hemoglobin concentration should be based upon the MCHC, because the latter value corrects for cell volume.

C. Mean corpuscular hemoglobin concentration (MCHC) represents the average Hb concentration per average erythrocyte in grams of Hb/100 mL of erythrocytes

1. Hb concentration (pg) × 100] ÷ Hct (%) = MCHC (g/dL)
2. The MCHC is the most accurate of the RBC indices because its calculation does not necessarily require the RBC count. However, if the Hct is a calculated value (as is the case in automated hematology analyzers), the accuracy of the MCHC may decrease.
3. The MCHC is used in the classification of anemias.
4. Factors affecting the MCHC
a. Increased MCHC is usually the result of *in vitro* or *in vivo* hemolysis or treatment with Oxyglobin®. Both intra- and extracellular Hb are measured in the hemoglobin procedure, but the formula assumes all Hb is intracellular, giving a false high value (Case 3).
b. A true increase in MCHC does not normally occur; increased concentrations of hemoglobin cannot be produced within the cell.
c. Reticulocytes do not have their full component of hemoglobin; therefore, the MCHC may be decreased in reticulocytosis (Cases 1, 2).
d. Hypochromia (i.e., low MCHC) occurs in some cases of iron deficiency (Case 5).
(1) Iron-deficient cats may not have hypochromic erythrocytes.
(2) The MCHC may not be low in iron-deficient dogs when measured on some electronic counters.

D. Red cell distribution width (RDW)

1. This erythrocyte parameter can be determined by some automated cell counters.
2. The RDW is the coefficient of variation of the red cell volume distribution and is calculated by the formula $RDW = (SD_{MCV} \div MCV) \times 100$. It is an index of the degree of anisocytosis or variation in size of the erythrocytes.
3. Anemias with significant microcytosis or macrocytosis have an increased RDW. Reticulocytosis may result in an increased RDW.

III. PERIPHERAL BLOOD SMEAR

A. Staining and examination of the smear

1. New methylene blue (NMB). A drop of NMB between a coverslip and an air-dried blood smear gives a rapid, meaningful stain but is a nonpermanent preparation. Acidic groups stain blue (i.e., nuclear DNA and RNA, cytoplasmic RNA, and basophil granules). Depending upon the distance

www.Ebook777.com

between the acidic groups, a blue or purple color may result. Eosinophil granules are unstained. The reticulum of reticulocytes stains blue, but reticulocytes are best stained by mixing equal parts of NMB and blood, holding at room temperature for 10 minutes, and then making the smear.

2. Romanowsky stains (e.g., Wright's stain, Diff-Quik®, Hemacolor®, etc.). These polychromic preparations stain certain acidic groups blue (RNA) to purple (mast cell and basophil granules and nuclear DNA), whereas basic groups stain red to orange (proteins, eosinophil granules).

3. Systematic evaluation of the stained blood smear

a. Low magnification. Select a thin area of the smear where the cells are evenly distributed, and look for the following features:

(1) RBC rouleaux formation (described below)

(2) RBC agglutination (described below)

(3) Platelet aggregation, especially at the feathered edge of the smear (Chapter 4)

(4) Relative number of leukocytes

b. High-dry magnification. Confirm observations made at low magnification, and:

(1) Note the concentration of leukocytes and obtain an impression as to whether the white blood cell (WBC) count is decreased, within the reference interval, or increased.

(2) Calculate a differential leukocyte count. This usually can be done at high-dry magnification, but certain cells may require oil immersion magnification for identification.

(3) Look for nucleated erythrocytes (nRBCs) and polychromasia (reticulocytes).

c. Oil-immersion magnification

(1) Examine the erythrocyte morphology (described below).

(2) Conduct the differential leukocyte count at this magnification if difficulty is encountered at high-dry magnification.

(3) Examine leukocyte morphology (Chapter 2).

(4) Estimate the adequacy of platelet numbers and evaluate their morphology (Chapter 4).

B. Erythrocyte morphology (Figure 1.7)

1. Normal morphology

a. Canine erythrocytes average 7 µm in diameter, are uniform in size, and have central pallor (biconcave disk).

b. Feline erythrocytes average 5.8 µm in diameter, have mild anisocytosis (i.e., variation in size), and exhibit very slight central pallor. Crenation is commonly observed. Howell-Jolly bodies (nuclear remnants) occur in up to 1% of the erythrocytes. Rouleaux formation also may be present.

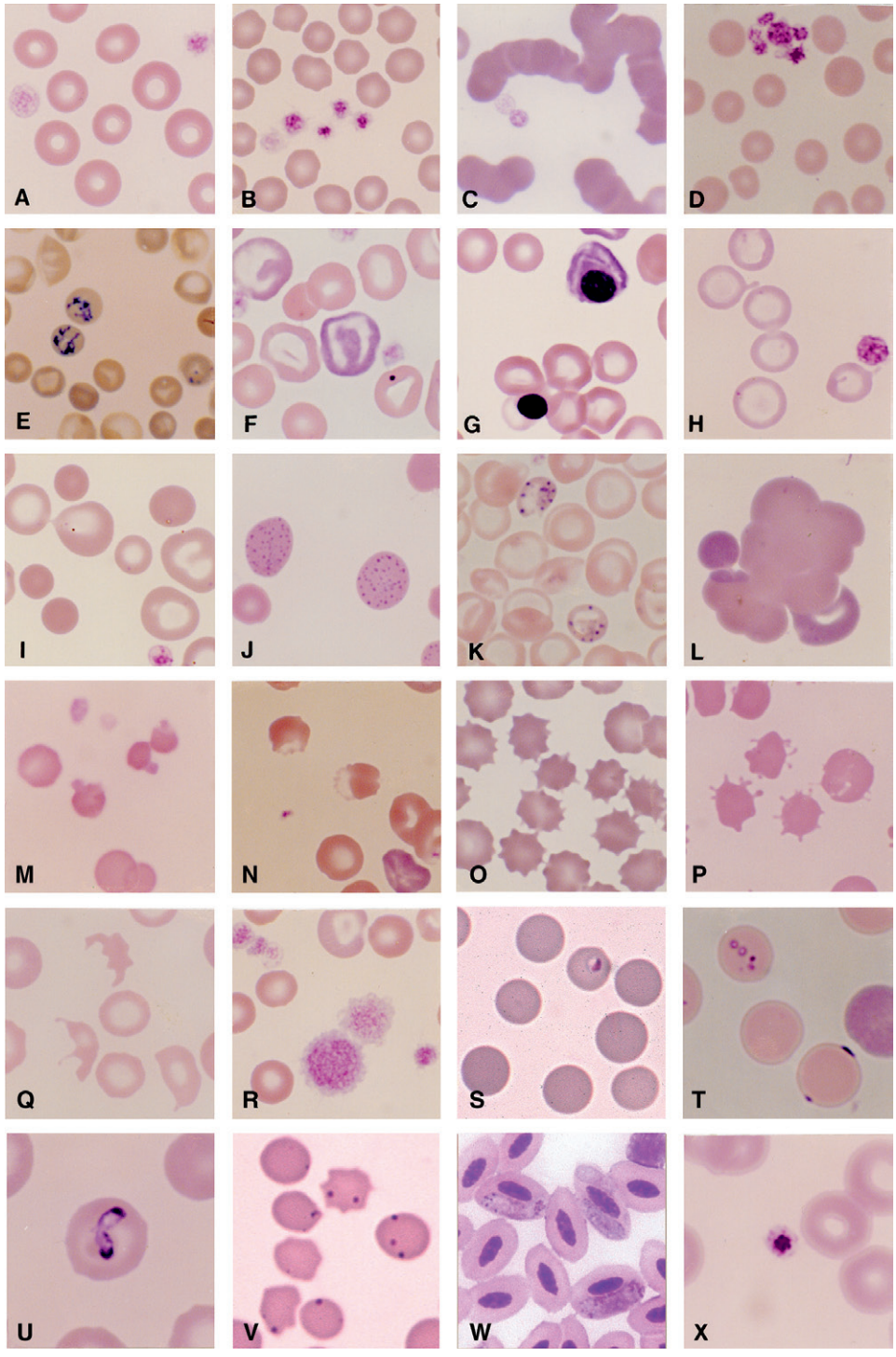
c. Bovine erythrocytes average 5.5 µm in diameter. Anisocytosis is common, and central pallor is usually slight. Crenation is common.

d. Equine erythrocytes average 5.7 µm in diameter and lack central pallor. Rouleaux formation is common.

e. Porcine erythrocytes average 6 µm in diameter and often exhibit poikilocytosis.

FIGURE 1.7. Erythrocyte and platelet morphology. A. canine erythrocytes and platelets; B. feline erythrocytes and platelets; C. equine erythrocytes in rouleaux and platelets; D. bovine erythrocytes and platelets; E. canine reticulocytes (new methylene blue stain); F. polychromasia, leptocytes, and Howell-Jolly body (dog); G. metarubricytes (dog); H. hypochromasia (iron deficiency, dog); I. spherocytes (immune-mediated anemia, dog); J. basophilic stippling (regenerative anemia, cow); K. basophilic stippling (lead toxicosis, dog); L. autoagglutination (immune-mediated anemia, dog); M. Heinz bodies (red maple toxicosis, horse); N. Heinz bodies in erythrocyte ghosts (acetaminophen toxicosis, cat); O. eccentrocytes (onion toxicosis, dog); P. keratocyte (dog); Q. echinocytes (dog); R. acanthocytes (dog); S. schistocytes (dog); T. macroplatelet (shift platelet; dog); U. *Mycoplasma haemofelis* (formerly *Hemobartonella felis*; cat); V. *Babesia canis* (dog); W. *Anaplasma marginale* (cow); X. *Anaplasma platys* (formerly *Ehrlichia platys;* dog) (Wright-Leishman stain unless indicated).

Free ebooks ==> www.Ebook777.com



www.Ebook777.com

f. Ovine erythrocytes are similar to those of the cow but smaller. The average diameter is 4.5 µm.

g. Caprine erythrocytes are the smallest blood cells of the domestic animals. They are usually less than 4 µm in diameter. Anisocytosis and poikilocytosis are common.

h. Camelidae (camel, llama, alpaca, etc.) erythrocytes are thin (approximately 1.1 µm) and ellipsoidal in shape with an average diameter of 6.5 µm, and usually have high erythrocyte counts.

i. Avian erythrocytes are oval and nucleated with an average size 12 × 6 µm.

2. Rouleaux formations are groups of erythrocytes resembling stacks of coins. The degree of rouleaux tends to positively correlate with the erythrocyte sedimentation rate (ESR), and is usually associated with an altered surface membrane charge (zeta potential). The intensity of this charge can be a species characteristic or the result of disease. Rouleaux is common in horses that have a decreased membrane charge in health. In certain diseases, normal membrane surface charge may be partially masked by excess protein (hyperfibrinogenemia, hyperglobulinemia) that decreases the repelling negative surface charges of the erythrocytes. Rouleaux and an increased ESR will be observed. Microscopically, rouleaux can be distinguished from autoagglutination by its dispersion in wet mounts when blood is diluted with physiologic saline solution.

a. Marked rouleaux formation is common in equine blood in health, but may be absent in the blood of severely anemic or cachectic horses.

b. Moderate and mild rouleaux may be present in feline and canine blood in health, respectively. Marked rouleaux may be observed during inflammatory and neoplastic diseases.

c. Rouleaux formation is rare in ruminant blood in health and disease.

3. Agglutination is a grape-like aggregation of erythrocytes occurring in some blood specimens of animals with immune- (antibody-) mediated anemia. Occasionally, it may be observed grossly (on the sides of the blood collection tube) or microscopically (in an unstained wet mount or on a stained blood smear). Agglutination is present if the erythrocytes remain clumped when blood is diluted 50:50 to 10:90 with physiologic saline solution and viewed microscopically as a wet mount. In anemic animals, agglutination is an indicator of an antibody-mediated effect, but the absence of agglutination does not exclude immune-mediated anemia.

4. Anisocytosis is variation in the size of erythrocytes because of the presence of macrocytes and/or microcytes among normocytes (normally-sized erythrocytes; Cases 1, 2, 5).

5. Macrocytes are large erythrocytes. Reticulocytes are usually macrocytic and polychromatophilic (light blue-gray color when using Wright's stain). Normochromic macrocytes may occur in certain conditions (e.g., macrocytosis of Poodles, FeLV infections, preleukemia of cats and dogs, erythroid aplasia of cats, and vitamin B_{12} deficiency of Giant Schnauzers). If significant numbers of macrocytes are present, the MCV may be increased.

6. Microcytes are small erythrocytes. They may be observed in iron and pyridoxine deficiency anemias in association with a low MCV. Microcytes can include cell remnants in Heinz body and fragmentation anemias. Microcytes also are associated with portosystemic shunts (PSS) and hyponatremia. Finally, microcytes may be observed in healthy Asian breeds of dogs (Akita, Chow Chow, Shar Pei, and Shiba Inu).

7. Spherocytes, associated with immune-mediated anemias, have a decreased MCV as a result of a decreased membrane surface area. Spherocytes are globoid because the remaining smaller cell membrane must enclose a normal amount of hemoglobin. Because spherocytes do not flatten well on the blood smear, they appear smaller than normochromic, biconcave disk erythrocytes.

8. Polychromasia refers to the blue-gray erythrocytes with residual RNA that are generally large (macrocytic) and seen on routinely stained blood smears. Polychromatophilic erythrocytes (as observed with Romanowsky stains) are synonymous with reticulocytes (as observed with NMB stain). Increased numbers of these cells are associated with increased erythropoietic activity and an attempted regeneration in response to anemia (Cases 1, 2, 5, 25). The degree of regeneration depends on the number of polychromatic erythrocytes (reticulocytes) relative to the degree of

Free ebooks ==> www.Ebook777.com

anemia. A few polychromic cells are normal in the dog and cat, less common in cattle, and not usually seen in horses (in health or anemia).

9. Hypochromia is decreased cytoplasmic staining intensity and increased central pallor of the erythrocyte caused by insufficient Hb within the red cell. The most common cause of hypochromia is iron deficiency, but it can also occur with lead toxicosis via inhibition of hemoglobin synthesis. Hypochromia in avian blood smears has been observed with lead toxicosis and inflammation.

10. Poikilocyte is a general term for an abnormally shaped erythrocyte. Blood smears should be submitted with CBC specimens to prevent artifactual alterations in cellular shape if there will be a substantial lag time between blood collection and analysis. Poikilocytes may be seen in young healthy calves and goats (due to structural Hb switching), as well as pigs of any age. Poikilocytes are considered an abnormality in other species where they can arise from trauma to the erythrocyte membrane associated with turbulent blood flow or intravascular fibrin deposition. Poikilocytes may be removed prematurely from circulation, leading to hemolytic anemia. Specific types of poikilocytes include the following:

a. Echinocytes are spiculated erythrocytes with many evenly spaced, uniform projections. Type I echinocytes contain spicules on the periphery of the erythrocyte. These are crenated erythrocytes that are an *in vitro* artifact associated with changes in temperature, pH, drying, or other interactions between the blood and smear preparation. Type II and III echinocytes (Burr cells) have spicules covering the entire surface of the rounded erythrocyte which are attributed to altered/fluxing electrolytes with expansion of the outer layer of the cell membrane. They also have been observed in uremia, electrolyte depletion, lymphoma, doxorubicin toxicity, and glomerulonephritis.

b. Keratocytes (helmet cells) are erythrocytes with one or two projections that form a ruptured vesicle. These abnormalities often result from oxidative damage to the erythrocyte membrane, as listed for Heinz body formation.

c. Schistocytes (schizocytes) are irregular erythrocyte fragments that result from shearing by intravascular fibrin or by turbulent blood flow within the vasculature (Case 11). Schistocytes are associated with disseminated intravascular coagulation (DIC), hemangiosarcoma, glomerulonephritis, congestive heart failure, myelofibrosis, chronic doxorubicin toxicosis, and vasculitis, to name a few conditions.

d. Acanthocytes are spiculated erythrocytes with two or more irregular, often blunted, projections. These cells are thought to form as a result of altered lipid:cholesterol ratios in the erythrocyte membrane. In animals, acanthocytes are associated with hemangiosarcoma (especially involving the liver), glomerulonephritis, lymphoma, and liver diseases.

e. Fusocytes are elongated erythrocytes that are seen in healthy Angora goats.

f. Elliptocytes are oval cells that are seen in healthy camelids. A rare hereditary disease in dogs with 4.1 band deficiency of the erythrocyte membrane cytoskeleton has been reported to have elliptocytosis. Occasionally, elliptocytes may be observed in iron deficiency.

g. Dacryocytes are teardrop-shaped erythrocytes that may result from the inability of the erythrocyte to return to its pre-existing shape after deforming in the blood vessels (decreased deformability). This change may be related to alterations in cytoskeleton proteins. If the "tails" of the dacryocytes are all in the same direction, this may be an artifact of blood smear preparation. Dacryocytes are observed in blood smears of llamas with iron deficiency anemia.

h. Leptocytes are thin cells with an increased membrane:volume ratio; they may appear folded due to the excess membrane. Leptocytes have been associated with portosystemic shunts (Case 13). Polychromatophilic erythrocytes (reticulocytes) may appear as leptocytes due to increased cell membrane.

i. Target cells (codocytes) are a type of leptocyte that are bell-shaped, but resemble a target on smears due to the distribution of Hb centrally and peripherally in the cell. Formation of target cells may occur by increasing the amount of membrane via lipid and cholesterol insertion or

www.Ebook777.com

by decreasing cytoplasmic volume as in hypochromia. Target cells may be associated with liver disease, iron deficiency anemia, and reticulocytosis.

j. Stomatocytes are a type of leptocyte that are bowl-shaped with oval areas of central pallor on blood smears. This change in shape results from expansion of the inner layer of the cell membrane. These cells are observed in hereditary stomatocytosis of Alaskan Malamutes, Drentse Partrijshond, and Miniature Schnauzers. Stomatocytes also can be artifacts in the thick area of the smear.

k. Spherocytes are small dark microcytes that lack central pallor and have a reduced amount of membrane per unit volume. They are readily detected only in the dog because of the normal abundance of central pallor. Spherocytes are observed most frequently in immune-mediated hemolytic anemias (Case 2), but may also be seen following transfusions and in some stages of Heinz body anemia. They result from partial phagocytic removal of antibody-coated membrane or "pitting" of Heinz bodies. Spherocytes are prematurely removed from circulation by splenic macrophages because of their reduced ability to deform (i.e., loss of cell flexibility) and to traverse the splenic microvasculature.

11. Basophilic stippling represents punctate aggregation of residual RNA in Romanowsky-stained (Wright- or Diff-Quik®-stained) cells. This often occurs in anemic sheep and cattle, and occasionally in feline anemia. It has the same significance as polychromasia (regenerative anemia), and can be an appropriate response during anemia. Basophilic stippling also may be an indication of lead toxicosis when accompanied by metarubricytosis with minimal polychromasia (an inappropriate response) in an animal with RBC indicators within reference values.

12. Howell-Jolly bodies are basophilic nuclear remnants within the cytoplasm of erythrocytes. These structures are observed more frequently in accelerated erythropoiesis or post-splenectomy.

13. The Heinz body is a round structure that protrudes from the membrane of the erythrocyte or appears as a small refractile spot in the cytoplasm. Heinz bodies are comprised of denatured, precipitated Hb caused by oxidation. They are often attached to the inner cell membrane (Case 3). Because Heinz bodies are derived from hemoglobin, they are the same color as the remainder of the cytoplasm and can be indistinct with Romanowsky staining. Following NMB staining, Heinz bodies appear as dark basophilic bodies. Heinz bodies alter the cell membrane and decrease erythrocyte deformability when traversing capillaries. They may result in intravascular hemolysis. The Heinz body itself may be removed by splenic macrophages, leaving a spherocyte. Cats are more susceptible to Heinz body formation (sometimes called erythrocyte refractile bodies or ER bodies in this species). Feline Hb has a larger number (8 to ten) of sulfhydryl groups that increase susceptibility to oxidation. Furthermore, the feline spleen is inefficient in removing these structures. Up to 10% of feline erythrocytes may contain Heinz (ER) bodies in health. In birds, Heinz bodies are smaller and more numerous within erythrocytes.

14. Eccentrocytes (hemi-ghost erythrocytes) are erythrocytes with the hemoglobin condensed in one portion of the cell, leaving a clear or blister-like area in the remaining portion of the cell. They are the result of oxidative injury with lipid peroxidation and cross-linking of the cell membrane (Case 3).

15. Nucleated erythrocytes (nRBCs) in the stained blood smear include metarubricytes, rubricytes, and earlier stages of erythroid development. The term "metarubricytosis" refers to the presence of any nRBCs and represents a premature release of these cells into the circulation. The release of nRBCs in disease may be classified as an appropriate or inappropriate response.

a. The release of nRBCs is an expected or appropriate response with an intense increase in erythropoiesis in strongly regenerative anemias. The nRBCs are accompanied by reticulocytosis (Cases 1, 2, 5). Erythropoietin also may stimulate release of nRBCs during hypoxia, unrelated to anemia.

b. For Camelidae, llamas often have high numbers of nRBCs in regenerative anemias accompanied by absence of or mild polychromasia.

Free ebooks ==> www.Ebook777.com

c. Metarubricytes may be inappropriately released in lead toxicosis, iron deficiency, copper deficiency, hemangiosarcoma, extramedullary hematopoiesis, myelophthisis, intervertebral disc syndrome, hereditary macrocytosis of Poodles, endotoxemia, bone marrow trauma, bone marrow necrosis, metastatic neoplasia of the marrow cavity, myelofibrosis, FeLV infection, myelodysplastic syndrome, and leukemia, especially erythremic myelosis in cats.

d. nRBCs may be seen in high numbers in healthy pigs, especially in piglets less than three weeks of age.

e. Nucleated erythrocytes normally occur in birds.

f. Nucleated erythrocytes have been reported in normal Miniature Schnauzers.

16. Erythroplastids are anucleate fragments of erythrocyte cytoplasm occasionally found in avian blood smears, while hematogones are free erythrocyte nuclei.

17. Parasites can occur within the erythrocyte (intracellular) or within depressions on the membrane surface (epicellular), or within the plasma (extracellular):

a. Intracellular parasites include *Hemoproteus* spp., *Leukocytozoon* spp., and *Plasmodium* spp. (birds); *Cytauxzoon felis, Babesia cati, B. felis* (cats); *Anaplasma marginale, A. centrale* (cattle); *Babesia bovis, B. bigemina* (cattle); *Theileria mutans, T. annulata* (cattle); *Theileria cervi,* (deer, elk); *Babesia canis, B. gibsoni* (dogs); *Babesia equi, B. caballi* (horses); and *Babesia ovis, B. motasi* (sheep).

b. Common epicellular parasites include *Trypanosoma johnbakeri* (birds), *Mycoplasma haemofelis* (formerly *Hemobartonella felis)* (cats), *M. haemocanis* (formerly *H. canis)* (dogs), *Mycoplasma haemosuis* (*formerly Eperythrozoon suis)* (pigs), *Eperythrozoon wenyoni* (cattle), and *Eperythrozoon* sp. (llamas).

c. Common extracellular parasites include filarids and trypanosomes.

(1) Microfilariae: *Dipetalonema reconditum* (dogs), *Dirofilaria immitis* (dogs, rarely cats), and *Setaria* sp. (horse).

(2) Trypanosomes: *Trypanosoma theileri, T. congolense, T. vivax* (cattle); *Trypanosoma cruzi,* (dogs); and *Trypanosoma brucei, T. evansi* (horses).

18. Distemper inclusions in canine erythrocytes are irregular to round to ring-shaped and stain magenta with Romanowsky and Diff-Quik® stains (the inclusions may stain blue with other rapid blood stains). They also may be observed in leukocytes. Distemper inclusions are transient.

IV. BLOOD RETICULOCYTE EVALUATION

A. Reticulocytes are immature anuclear erythrocytes in mammals that contain residual RNA and mitochondria, aggregated into a reticular pattern when stained with supravital stains (e.g., NMB stain) (Figure 1.7). Reticulocytes correspond to polychromatophilic erythrocytes observed in Romanowsky-stained preparations. Reticulocytes released prematurely in response to an anemia are larger and are called shift reticulocytes. Reticulocytes in birds are nucleated and occasionally may appear more rounded.

B. Quantitation of reticulocytes in circulation, by calculation of absolute reticulocyte numbers, is the best indicator of the bone marrow erythroid response to anemia. Newer hematology analyzers have the ability to segregate reticulocytes into age groups (time from release from bone marrow).

C. Species characteristics of reticulocytes

1. The dog has small numbers (up to 1%) of aggregate type reticulocytes (containing blue-stained aggregates with supravital stains) in health (Figure 1.7).

2. Two types of reticulocytes occur in the cat.

a. Aggregate reticulocytes are similar to those of other species and account for up to 0.4% of the erythrocytes in health. This type of reticulocyte is enumerated hematologically. Increased

www.Ebook777.com

aggregate reticulocytes reflect the current bone marrow response to anemia (i.e., increased erythropoiesis or regeneration). Reticulocytosis is less dramatic than in the dog.

b. Punctate reticulocytes (containing small, blue-stained dots) are derived from aged aggregate reticulocytes and persist for at least two weeks. Like aggregate reticulocytes, they are increased with increased erythropoiesis. Because punctate reticulocytes circulate for weeks and persist after the aggregate reticulocyte count has returned to the reference interval, their enumeration is of little benefit in assessing the current regenerative response. Punctate reticulocytes indicate a bone marrow response to anemia occurring as much as three to four weeks previously.

3. Reticulocytes are absent from the blood of ruminants in health because of the long erythrocyte lifespan, but increase modestly in responding anemias.

4. Reticulocytes are absent in the blood of horses in both health and regenerative anemias.

5. Reticulocytosis is a prominent feature of healthy suckling pigs, but is less dramatic in adults (1%). Reticulocyte counts increase in regenerative anemias.

6. Reticulocyte percentage in the blood of healthy birds is higher (4% to 5%) than mammals because of a short erythrocyte lifespan (approximately 35 days). Polychromasia is more prominent in younger birds than in adults but usually does not exceed 5%.

D. Means of reticulocyte enumeration

1. Reticulocyte percentage. Reticulocytes are counted in a NMB-stained blood smear and are expressed as the percentage of the total erythrocyte population. This parameter can overestimate the bone marrow response because:

a. Reticulocytes released from the bone marrow into the blood of anemic animals are mixed with fewer mature erythrocytes. Thus, a higher relative percentage of reticulocytes results.

b. Larger, more immature reticulocytes are released earlier (shift reticulocytes) in response to anemia. These reticulocytes persist longer in the blood because it takes them more time to mature. This results in a higher percentage of reticulocytes.

2. Corrected reticulocyte percentage

a. Formula: Observed reticulocyte percentage × (patient's Hct percentage ÷ "normal" Hct percentage) = corrected reticulocyte percentage

The "normal" hematocrit (Hct) is considered to be 45% in the dog and 37% in the cat.

b. This parame'ter corrects for (1a) above but not for (1b).

c. Corrected reticulocyte percentages greater than 1% in the dog and greater than 0.4% in the cat indicate bone marrow response to the anemic state. The corrected reticulocyte percentage has not been validated for use in other animal species.

3. Absolute reticulocyte count

a. Formula: Reticulocyte percentage (converted to a decimal figure) × (total RBC count/μL) = absolute reticulocyte count/μL

b. This parameter corrects for item 1a (above) but does not correct for the effect described in item 1b (above).

c. An absolute reticulocyte count greater than 80,000/μL in the dog and greater than 60,000 /μL in the cat indicates a regenerative response. Depending on the reduction in Hct, a very low Hct should have a proportionately higher absolute reticulocyte count.

d. The following are estimated degrees of regeneration for dogs:

(1) None = 60,000/μL
(2) Slight = 150,000/μL
(3) Moderate = 300,000/μL
(4) Marked = more than 500,000/μL

e. The following are estimated degrees of regeneration (aggregate reticulocytes) for cats:

(1) None = less than 15,000/μL
(2) Slight = 50,000/μL
(3) Moderate = 100,000/μL
(4) Marked = more than 200,000/μL

Free ebooks ==> www.Ebook777.com

f. Although not often used, similar estimates of regeneration for feline punctate reticulocytes include the following:

(1) None = less than 200,000/µL

(2) Slight = 500,000/µL

(3) Moderate = 1,000,000/µL

(4) Marked = 1,500,000/µL

4. Reticulocyte production index (RPI). This correction index is used in humans and also has been used in the dog and cat to correct for items 1a and 1b above. This parameter remains controversial because definitive correlation studies have not been done in animals.

a. RPI = observed reticulocytes (percentage) × [observed Hct (percentage) ÷ 45 (normal Hct)] × [1 + blood maturation time]

b. Blood maturation times of reticulocytes at various Hcts are 45% = 1 day, 35% = 1.5 days, 25% = 2 days, and 15% = 2.5 days.

c. RPI values equal the increase in erythrocyte production (e.g., RPI of 3 equals three times normal erythrocyte production). An RPI greater than 2 equals a regenerative response.

E. Interpretation of reticulocyte parameters

1. Absolute increases in reticulocytes indicate a responding bone marrow (regenerative anemia) and that the cause of the anemia is extra-marrow (i.e., hemorrhage or hemolysis).

2. Reticulocytosis is more intense in hemolytic than in external hemorrhagic anemias. Iron from disrupted erythrocytes is more readily available for erythropoiesis than the iron that is stored as hemosiderin.

3. Reticulocytosis does not become clearly evident until 48 to 72 hours after the occurrence of anemia. With normally responsive bone marrow, maximum reticulocytosis should be achieved within seven days. However, reticulocytosis may be delayed in animals with various systemic diseases and in aged animals.

4. Dogs have a greater reticulocyte response than cats.

5. Significant reticulocytosis does not develop in cattle with acute responding anemias until the Hct is very low.

6. Reticulocytosis does not occur in the horse with any type of anemia.

7. Healthy suckling pigs have high reticulocyte counts.

8. Healthy birds often have a higher reticulocyte count (approximately 4% to 5%) than mammals due to their short erythrocyte life span (25 to 40 days).

9. The lack of a reticulocyte response following anemia suggests that the bone marrow is not responding (nonregenerative anemia). This may be due to insufficient time for reticulocytosis to occur, a deficiency in the existing reticulocyte response, or defective erythropoiesis.

V. BONE MARROW EXAMINATION

A. Indications for examination

1. Nonregenerative or nonresponding anemia

2. Persistent neutropenia

3. Unexplained thrombocytopenia

4. Suspicion of hematopoietic neoplasia

5. Suspicion of osteomyelitis, infiltrative, or proliferative bone marrow disease

6. Fever of unknown origin

B. Technique

1. Bone marrow aspirates can be obtained from the iliac crest, trochanteric fossa, sternum, humerus, or rib of mammals using a special bone marrow or 18-gauge spinal needle. Aspirates are

www.Ebook777.com

obtained from the sternal ridge (keel) or tibiotarsus in birds. Other prominent long bones of birds are pneumatized and lack significant hematopoietic tissue.

2. Particle smears are preferred because they are less likely to have blood contamination.

3. Core biopsies, obtained with special needles, may be taken from the above locations and provide a better indication of overall cellularity than cytologic smears alone.

C. Examination of the stained bone marrow smear

1. Observe the relative number, size, and cellularity of the particles, including the proportion of adipocytes (approximately 50%) and hematopoietic progenitor cells (approximately 50%). The number and cellularity of particles is an estimation of the overall cellularity of the bone marrow; however, low-cellularity aspirates may occur with incomplete sampling of the bone marrow. Histologic examination of a bone marrow core biopsy is more accurate in assessing marrow cellularity and in detecting stromal reactions (e.g., myelofibrosis).

2. Note the adequacy of megakaryocyte numbers, maturity, and morphology. Immature megakaryocytes have blue, granular cytoplasm, whereas mature megakaryocytes have pink, granular cytoplasm.

3. The ratio of myeloid:nucleated erythroid cells (M:E ratio) may be estimated.

a. The M:E ratio is usually determined to assess the erythropoietic response to anemia. The WBC count or, more specifically, the neutrophil count, is needed for proper interpretation of the ratio.

b. If the WBC count is within the reference interval, any change in the M:E ratio is due to changes in the erythroid series.

c. A high M:E ratio in an anemic animal with a WBC count within the reference interval suggests erythroid hypoplasia.

d. A high M:E ratio in an anemic animal with an increased WBC count is more difficult to interpret because the increased ratio could result from myeloid (granulocytic) hyperplasia and/or erythroid hypoplasia.

e. A low M:E ratio in an anemic animal with a WBC count within or above the reference interval suggests early erythroid regeneration of ineffective erythropoiesis.

4. The relative percentages of the various stages of each series should be observed to assess maturation (Figure 1.5). In health, approximately 80% of the myeloid series should be metamyelocytes, bands, and segmenters (nonproliferating, maturation, storage pool); 90% of the erythroid series should be rubricytes and metarubricytes. A high percentage of immature forms suggests hyperplasia, neoplasia, or maturation abnormality of the respective series. In the myeloid series, depletion of the storage pool (which contains the more mature cells) can cause a shift toward immaturity.

5. Abnormal cells should be identified and described.

6. Perl's or Prussian blue staining for iron in bone marrow macrophages may be useful to distinguish iron deficiency anemia (decreased iron stores) from the anemia of chronic disease (increased iron stores). Perl's or Prussian blue staining detects hemosiderin (insoluble iron) but does not detect ferritin (soluble iron that is removed during staining). Hemosiderin usually is not observed in feline bone marrow.

VI. ANTIGEN AND ANTIBODY DETECTION

A. General concepts concerning RBC antigens and antibodies

1. In domestic animals, anti-erythrocyte antibodies are a consequence of transfusions, cross-placental transfer during pregnancy in the horse, vaccination with blood origin products, autoimmune phenomena, and natural occurrence in the cat.

2. Antigen identification is helpful in parentage testing, identifying potentially compatible blood donors (e.g., DEA-1.1-, 1.2-, and 1.3-negative dogs are best as blood donors), and identifying

Free ebooks ==> www.Ebook777.com

matings likely to lead to isoimmunization and subsequent hemolytic disease of newborn animals (neonatal isoerythrolysis) (e.g., Aa-negative mares bred to Aa-positive stallions are at greater risk with the second foal).

3. Agglutination and hemolytic tests with specific antisera for each antigen are used for identification. Some antigens (blood group subtypes) are more important than others in determining animals at risk of hemolytic disorders. Blood from animals with the following blood antigen types has the greatest potential to cause sensitization and subsequent reactions:

a. In the dog, DEA (dog erythrocyte antigen)-1.1 (Aa_1), DEA-1.2 (Aa_2), and DEA-1.3 (Aa_3) are highly immunogenic and will sensitize recipients. Anti-DEA 1.1 and anti-DEA 1.2 are not naturally occurring, and their presence in blood requires prior exposure to these antigens. Anti-DEA 1.1 and anti-DEA 1.2 antibodies result in hemolytic reactions. Naturally occurring anti-DEA-3 (Ba) antibodies result in early erythrocyte removal or hemolysis. Anti-DEA 5 (Da) and anti-DEA-7 (Tr) antibodies occur naturally and result in increased erythrocyte removal.

b. Antibodies in mare colostrum to Aa and Qa blood types are most frequently involved in neonatal isoerythrolysis.

c. Cattle have marked variability of erythrocyte antigens with 70 blood group factors.

d. Type A and B antibodies occur naturally in the cat. Type B cats with anti-A antibody may have life-threatening hemolytic reactions when transfused with type A blood. Type A cats with anti-B antibodies have early erythrocyte removal when transfused with type B blood.

B. Detection of anti-erythrocyte antibodies

1. Blood cross matching

a. These tests are used to detect antibodies and determine when blood may be safely transfused.

b. The major cross match tests erythrocytes of the donor against serum of the recipient. This is a test to detect antibodies in the recipient that will react with donor cells. Incompatibilities in the major cross match are clinically significant because of the systemic reaction caused by the massive lysis of transfused cells.

c. The minor cross match tests serum of the donor against erythrocytes of the recipient. This is a test to detect antibody in donor blood. Incompatibilities are not life threatening because the transfused antibody will be diluted in the recipient.

d. Incompatible cross matches usually indicate prior sensitization.

(1) Naturally occurring antibodies usually are not present in sufficient concentration in animals to cause transfusion reactions, except in A-negative (Type-B) cats, which have naturally occurring anti-A antibodies.

(2) An incompatible cross match, therefore, indicates prior sensitization, except in the cat.

e. An incompatible cross match is indicated by agglutination in most species; however, hemolysis predominates in the horse and cow.

2. Antiglobulin (Coombs') tests

a. Antiglobulin (Coombs') tests are used to confirm immune-mediated hemolysis of erythrocytes.

b. The direct antiglobulin test (DAT) detects antibody and/or complement attached to the membrane of the patient's washed erythrocytes.

(1) The antiglobulin reagent (Coombs' serum) may be prepared against any antibody type (IgG, IgM) or complement (C3) or may be a mixture (IgG and C_3). Reagents that test for one antibody or complement are monovalent. Reagents that test for multiple antibodies and/or complement are polyvalent.

(2) Species-specific Coombs' serum must be used (e.g., rabbit anti-canine IgG for the dog).

c. The indirect antiglobulin test detects anti-erythrocyte antibody in the serum of the patient. In this test, the patient's serum is tested against washed erythrocytes from the sire, offspring, or a prospective blood donor animal. Supernatant (whey) from colostral milk may be used instead of serum in postpartum mares or cows to detect potential reactions against the offspring's cells.

www.Ebook777.com

d. A newer cell-enzyme-linked immunosorbent assay (ELISA)-based method (direct enzyme-linked antiglobulin test or DELAT test) has been described and used successfully to detect antibodies in DAT-negative canine autoimmune hemolytic anemia (AIHA). IgG, IgM, IgA, and C_3 have been detected coating the RBC membrane in dogs; however, this test has been used primarily as a research tool.

ANEMIA: DIAGNOSIS AND CLASSIFICATION

Anemia is an absolute decrease in the Hct, Hb concentration, and/or RBC count. Relative anemia may occur when the plasma volume is expanded (e.g., excessive parenteral fluid administration, pregnancy, neonates) or when blood specimens are improperly obtained from intravenous fluid lines (pseudoanemia).

I. DETERMINATION OF THE CAUSE OF ANEMIA. AS WITH ANY DISEASE, THE DIAGNOSIS OF ANEMIA IS MADE FROM HISTORICAL INFORMATION, PHYSICAL FINDINGS, AND LABORATORY FINDINGS.

A. History. The following findings may be important:

1. Drug administration or vaccination
2. Exposure to toxic chemicals or plants
3. Familial or herd occurrence of disease
4. Recent transfusions or colostral ingestion
5. Age at onset of clinical signs
6. History of prior blood disorder
7. Diet
8. Breed, signalment
9. Prior pregnancy of dam
10. Reproductive status (intact versus neutered)

B. Physical findings

1. Clinical signs suggesting the presence of anemia are related to decreased oxygen transport capacity of the blood and physiologic adjustments to increase the efficiency of the erythron and reduce the workload on the heart. Typical clinical signs include the following:

- **a.** Pale mucous membranes
- **b.** Weakness, loss of stamina, exercise intolerance
- **c.** Tachycardia and polypnea, particularly after exercise
- **d.** Syncope, depression
- **d.** Increased sensitivity to cold
- **e.** Heart murmur caused by reduced viscosity and increased turbulence of blood flow
- **f.** Weak or fluttering pulse
- **g.** Shock, if one-third of the blood volume is lost rapidly

2. Icterus, hemoglobinuria, hemorrhage, melena, petechiae, or fever may be observed, depending on the pathophysiologic mechanism involved.

3. Clinical signs are less obvious if the onset of anemia is gradual and the animal has adapted to the decreased erythrocyte mass and lowered oxygen transport capability. In this scenario the anemia upon presentation can be profound (Hct of less than 10%).

C. Laboratory findings. Laboratory confirmation is necessary because anemias are not always accompanied by typical clinical signs. Mild anemia often is diagnosed from the laboratory data of a sick animal when its presence was not previously suspected.

Free ebooks ==> www.Ebook777.com

1. The Hct is the easiest, most accurate method to detect anemia. The Hct value should be interpreted with knowledge of the patient's hydration status and with consideration of any possible influence of splenic contraction.
2. Hb concentration and RBC count may be used to further classify the anemia but usually are not needed to confirm its presence.
3. Other laboratory procedures may be used to further characterize the anemia and arrive at a specific diagnosis. These procedures are discussed below and earlier in this chapter.

II. CLASSIFICATION

The cause of the anemia should be identified when possible because the term "anemia" per se does not constitute a definitive diagnosis. Classification schemes are used for definitive diagnosis, although a single classification may not be entirely satisfactory.

A. Classification according to size (MCV) and Hb concentration (MCHC) of the erythrocyte
1. The MCV categorizes the anemia as normocytic, macrocytic, or microcytic. The average erythrocyte volume is within the reference interval, increased, or decreased, respectively.
2. The MCHC categorizes the anemia as normochromic, hypochromic, or hyperchromic. The Hb concentration is within the reference interval if the erythrocytes are normochromic. Hb concentration is decreased if the erythrocytes are hypochromic. Hyperchromasia is most often the result of erythrocyte hemolysis or Oxyglobin® administration, because erythrocytes do not overproduce hemoglobin. In rare circumstances, spherocytosis may be associated with hyperchromasia because of decreased erythrocyte volume.

B. Classification according to bone marrow response
1. Regenerative anemia
a. The bone marrow actively responds to the anemia by increasing production of erythrocytes.
b. Findings that denote regeneration of erythrocytes
(1) Polychromasia
(2) Reticulocytosis with anisocytosis and increased RDW
(3) Macrocytosis (increased MCV) and hypochromasia (decreased MCH and MCHC) associated with reticulocytosis
(4) Basophilic stippling of erythrocytes in ruminants
(5) Hypercellular bone marrow with a decreased M:E ratio due to erythroid hyperplasia
c. Species that are capable of the highest maximal reticulocyte response exhibit the most intense regeneration; the Hct returns to the reference interval more rapidly. In decreasing order, the ability of a species to mount a regenerative response is bird, dog, cat, cow, horse.
d. The presence of regeneration suggests an extramarrow etiology, implying loss (e.g., hemorrhage) or lysis (e.g., Heinz bodies, immune-mediated, or fragmentation) of erythrocytes of sufficient duration (two to three days) for a regenerative response to be evident in the blood.
e. Bone marrow examination is rarely necessary to detect erythrocyte regeneration; however, erythroid hyperplasia should be evident if aspirates are taken.
f. Regeneration is difficult to detect in the horse because reticulocytes are not released into the blood. An increased MCV and RDW may suggest a regenerative response. Bone marrow examination may be helpful but is not always conclusive; erythroid hyperplasia and increased numbers of reticulocytes may be observed in the aspirates.
g. Examples of regenerative anemias include hemolysis, hemorrhage, or regeneration after the cause of a nonregenerative anemia has been resolved.
2. Nonregenerative anemia
a. Nonregenerative anemia suggests the lack of an erythroid response in the bone marrow. Lack of response could be the result of inadequate time for erythropoiesis to occur as well as

www.Ebook777.com

conditions such as chronic inflammation, renal disease, and endocrine disorders. Horses do not release reticulocytes into blood; therefore, all anemias appear nonregenerative in this species.
b. Polychromasia, reticulocytosis, and basophilic stippling (ruminants) are inadequate to absent.
c. During the first two to three days after the onset of peracute or acute hemorrhage or hemolysis, the anemia may appear nonregenerative.
d. Bone marrow examination occasionally may reveal the pathophysiologic mechanism or etiology of some cases of nonregenerative anemia.
e. Examples of nonregenerative anemia include: anemia of inflammatory disease (AID), renal failure, iron deficiency anemia, aplastic anemia, pure red cell aplasia, and endocrine disorders.
f. The cause of anemia may be multifactorial, which could affect the regenerative response. For example, acute hemorrhage may be minimally regenerative or nonregenerative if the underlying cause is an inflammatory condition resulting in AID.

C. Classification according to major pathophysiologic mechanisms
1. Blood loss (hemorrhagic anemia)
2. Accelerated erythrocyte destruction (decreased erythrocytic life span) by intra- or extravascular hemolysis
3. Reduced or defective erythropoiesis

ANEMIA FROM BLOOD LOSS (HEMORRHAGIC ANEMIA) (TABLE 1.1)

I. CHARACTERISTICS OF ACUTE BLOOD LOSS (CASES 1, 25)

A. Clinical findings
1. Direct visual evidence of hemorrhage usually is present, but occult hemorrhage may occur. When laboratory findings suggest hemorrhagic anemia and direct evidence of hemorrhage cannot be found, sources of occult bleeding such as gastrointestinal hemorrhage should be considered. Thrombocytopenia and clotting test abnormalities indicate the potential for hemorrhage. Thrombocytopenia alone rarely results in hemorrhagic anemia. Autotransfusion may accompany hemorrhage into body cavities.

TABLE 1.1.
CAUSES OF BLOOD LOSS.

Acute hemorrhage	Chronic hemorrhage
GI ulcers	GI ulcers, neoplasms
Hemostasis defects	Hematuria
Bracken fern toxicosis	Hemophilia
Disseminated intravascular coagulation	Neoplasia
Factor X deficiency	Gastrointestinal neoplasms
Hemophilia A and B	Vascular neoplasms
Rodenticide toxicosis	Parasitism
Sweet clover toxicosis	Ancylostomiasis
Neoplasia	Coccidiosis
Splenic hemangiosarcoma	Fleas, ticks, lice
Splenic hemangioma	Hemonchosis
Thrombocytopenia	Strongylosis
Trauma	Vitamin K deficiency
Surgery	

Free ebooks ==> www.Ebook777.com

2. Clinical signs depend upon the amount of blood lost, period of time during which bleeding occurred, and site of hemorrhage. Hemorrhage from multiple sites or delayed onset of hemorrhage at sites of vascular intervention suggest clotting abnormalities (Chapter 4).

B. Laboratory findings

1. The Hct initially is within the reference interval because all blood components (i.e., cells and plasma) are lost in similar proportions. The animal may be in hypovolemic shock if more than 33% of the blood volume is lost rapidly.

2. Splenic contraction delivers high-Hct splenic blood (Hct = 80%) into the circulation, temporarily increasing the Hct.

3. Blood volume subsequently is restored by the addition of interstitial fluid beginning two to three hours after the onset of hemorrhage and proceeding for 48 to 72 hours. This fluid shift causes dilution of the erythrocyte mass and laboratory signs of anemia (i.e., reduced Hct, RBC count, and Hb concentration) become evident. Hypoproteinemia (decreased plasma protein concentration) also may be observed.

4. Platelet numbers usually increase during the first few hours after hemorrhage. Persistent thrombocytosis may suggest continued blood loss.

5. Neutrophilic leukocytosis commonly occurs approximately three hours post-hemorrhage.

6. Signs of increased erythrocyte production (e.g., polychromasia, reticulocytosis) become evident by 48 to 72 hours and reach maximum values approximately seven days after the onset of hemorrhage. Erythroid hyperplasia is evident in bone marrow aspirates and precedes changes in the blood.

7. Plasma protein concentration begins to increase within two to three days and returns to the reference interval before Hct, RBC count, and Hb concentrations normalize.

8. The entire hemogram returns to reference intervals in one to two weeks in the dog following a single, acute, hemorrhagic episode. If reticulocytosis persists longer than two to three weeks, continuous bleeding should be suspected.

9. Thrombocytopenia and subsequent hemorrhage may occur with primary bone marrow failure; the anemia in these cases appears nonregenerative.

II. CHARACTERISTICS OF CHRONIC BLOOD LOSS (CASE 5)

A. Clinical findings

1. Anemia develops slowly and hypovolemia does not occur.

2. The Hct can reach low values before clinical signs of anemia become obvious because the slow onset of anemia allows for physiologic adaptations.

B. Laboratory findings

1. A regenerative response occurs but is usually less intense than with acute blood loss.

2. Hypoproteinemia usually is observed.

3. Persistent thrombocytosis may be evident.

4. Iron deficiency anemia, characterized by microcytosis and hypochromasia, may develop over time as body iron stores are depleted.

III. DIFFERENTIAL FEATURES OF ANEMIAS CAUSED BY EXTERNAL AND INTERNAL HEMORRHAGE

A. External blood loss, including gastrointestinal bleeding, prevents reutilization of certain components such as iron and plasma protein. These may be reabsorbed and recycled following internal hemorrhage.

www.Ebook777.com

B. Internal hemorrhage may be less severe and more intensely regenerative. Following internal hemorrhage, some erythrocytes are reabsorbed by lymphatic vessels (autotransfusion), particularly when hemorrhage occurs into body cavities. Remaining erythrocytes are lysed or phagocytosed. The iron and amino acids from catabolized proteins are recycled.

ANEMIA FROM ACCELERATED ERYTHROCYTE DESTRUCTION (HEMOLYTIC ANEMIA) (TABLE 1.2)

I. CHARACTERISTICS OF HEMOLYTIC ANEMIA (CASES 2, 3)

A. Clinical findings

1. Clinical signs of hemorrhage are absent.

2. In acute hemolytic anemia, clinical signs related to the severity of anemia may be dramatic because compensatory mechanisms develop more slowly.

3. Icterus may be seen in acute, severe hemolytic anemia.

4. Hemoglobinuria and hemoglobinemia (red plasma) are seen if significant intravascular hemolysis occurs.

5. Extravascular hemolysis is much more common than intravascular hemolysis.

B. Laboratory findings

1. Reticulocyte counts characteristically are higher in hemolytic anemias than in external hemorrhagic anemias. Iron from hemolyzed erythrocytes is more readily available for erythropoiesis than is storage iron or hemosiderin. When iron-containing blood is lost externally, body storage iron must be mobilized for increased erythropoiesis, delaying regeneration.

2. Plasma protein concentration is within the reference interval or increased (hyperproteinemia). Hemoglobinemia may be present with intravascular hemolysis, resulting in artificial hyperchromasia (increased MCH and MCHC) and hyperproteinemia.

3. Neutrophilic leukocytosis and monocytosis may occur.

4. Evidence of Hb degradation (e.g., hyperbilirubinemia, hemoglobinuria) may be present.

5. Abnormal erythrocyte morphology (e.g., Heinz bodies, erythrocytic parasites, spherocytes, or poikilocytes) (Figure 1.7) may suggest the mechanism of hemolysis.

II. DIFFERENTIATION OF THE CAUSES OF HEMOLYTIC ANEMIAS

A helpful approach in diagnosing hemolytic anemias is to identify the site and mechanism of erythrocyte destruction. Hemolysis can occur outside of the blood vessels following phagocytosis of erythrocytes by macrophages (extravascular hemolysis), or hemolysis can occur within blood vessels (intravascular hemolysis). Hemolysis can occur both extra- and intravascularly; however, erythrocyte destruction usually predominates at one of these sites.

A. Extravascular hemolysis (phagocytosis) (Case 2) Erythrocytes are sequestered in spleen or liver, where they are phagocytized or lysed. Hemoglobin is catabolized at the site of destruction.

1. Mechanisms of extravascular hemolysis

a. Antibody and/or C_3b mediated (Case 2)

(1) Antibody binds to an erythrocyte membrane antigen or other antigen (including haptens) tightly adsorbed to the erythrocyte membrane. Nonerythrocyte antigen-antibody complexes may be non-specifically adsorbed to the cell membrane. Glycophorins, band 3, and spectrin are the membrane antigens that usually are recognized by antibodies.

(2) C_3b is fixed into the erythrocyte membrane by the antigen-antibody reaction. Adsorbed immune complexes may fix C_3b and then elute from the cell, leaving only C_3b deposited on

Free ebooks ==> www.Ebook777.com

TABLE I.2.
CAUSES OF ACCELERATED ERYTHROCYTE DESTRUCTION.

Intravascular hemolysis*	Extravascular (phagocytic) hemolysis*
Bacteria	RBC parasites
Clostridium hemolyticum	*Anaplasma* spp.
Cl. novyi	*Cytauxzoon* spp.
Cl perfringens	*Mycoplasma* (formerly *Eperythrozoon*) spp.
E. coli (hemolytic uremic syndrome)	*Mycoplasma* (formerly *Hemobartonella*) spp.
Leptospira spp.	*Theileria* spp.
RBC parasites	*Trypanosoma* spp.
Babesia spp.	Immune-mediated
Chemicals and plants	Autoimmune hemolytic anemia (dogs, cats)
Oxidants	Equine infectious anemia virus
Acetaminophen	*Anaplasma* spp. (formerly *Ehrlichia*)
Benzocaine	Feline leukemia virus
Brassica spp.	Lupus erythematosus
Copper, molybdenum deficiency	Hemangiosarcoma
Onions	Hematopoietic neoplasia
Phenothiazine	Penicillin
Phenazopyridine	RBC parasites
Propylene glycol	*Sarcocystis* spp.
Red maple	Intrinsic erythrocytic defects
Rye grass	Erythrocytic porphyria
Vitamin K	Hereditary stomatocytosis
Cephalosporins	Pyruvate kinase deficiency (dogs)
Ricin (castor bean)	Fragmentation
Snake venoms	Disseminated intravascular coagulation
Zinc	Heartworms
Immune-mediated	Hemangiosarcoma
Autoimmune hemolytic anemia (horses, cattle)	Vasculitis
Hemolytic disease of newborn (neonatal isoerythrolysis)	Hemophagocytic syndrome
Incompatible transfusions	Hypersplenism
Hypo-osmolality	Malignant histiocytosis
Selenium deficiency (cattle)	
Cold hemoglobinuria	
Hypotonic fluids	
Water intoxication	
Fragmentation	
Vena caval syndrome	
Hypophosphatemia	
Hyperalimentation	
Postparturient hemoglobinuria	
G-6-PD deficiency	
GSH deficiency (sheep)	
Hepatic failure (horses)	
Phosphofructokinase deficiency (dogs)	

*Many of the conditions listed in this table have both intravascular and phagocytic components, but are listed by the predominant type of hemolysis.

www.Ebook777.com

the membrane. The erythrocyte is an innocent bystander but is subsequently targeted for phagocytic removal.

(3) Macrophages have receptors for both the Fc component of antibody and C_3b. These receptors facilitate the recognition and attachment of macrophages to erythrocyte membranes that are coated with antibody and/or C_3b. Affected erythrocytes are completely or partially phagocytosed. Spherocytes are formed by partial phagocytosis with subsequent resealing of the erythrocyte's membrane. Because more membrane is removed than cell contents, the spherocyte appears small and round, and lacks central pallor (Figure 1.7).

(4) Immune-mediated hemolytic anemias may be caused by the following mechanisms when erythrocytes, and rarely erythroid precursors, are destroyed by a Type II hypersensitivity response.

(a) Unknown mechanisms, termed idiopathic autoimmune hemolytic anemia (AIHA)

(b) Infectious agents such as FeLV, EIA virus, *Ehrlichia* spp., or *Mycoplasma haemofelis* (formerly *Hemobartonella felis*)

i) These agents may alter the erythrocyte membrane, exposing hidden antigens to which the host produces antibody.

ii) Some pathogens form immune complexes that adsorb to the cell and fix complement (C_3b).

iii) Cross-reacting antibody may be formed in response to infection.

(c) Some drugs, such as penicillin, adsorb to the erythrocyte membrane and act as haptens in the production of anti-drug antibodies.

(d) Alterations in the immune system

i) Disturbances in T-cell function may disrupt immune regulation. T-cells from AIHA or AIHA-related sibling dogs have a greater degree of reactivity.

ii) Coombs' positive anemia has been observed in some lymphoid malignancies, protozoal diseases, rickettsial diseases, *Mycoplasma haemofelis (formerly Hemobartonella felis)*, FIV, FIP, and other chronic inflammatory diseases.

(e) Immune-mediated anemia also may occur as a paraneoplastic syndrome with lymphoma and plasma cell myeloma.

(5) The direct antiglobulin or Coombs' test detects warm-active IgG alone, IgG plus C_3, C_3 alone, and, rarely, cold-active IgM on the erythrocyte cell membrane. Cold-reactive IgM may fix complement in the absence of IgG. This cold agglutinin disease is often associated with autoagglutination, intravascular hemolysis, acute onset, and severe clinical signs. A newer and more sensitive direct-enzyme linked antiglobulin test (DELAT) has identified multiple immunoglobulin types (IgG, IgM, IgA) and complement components (C_3) on the surface of the erythrocyte membrane. A synergistic effect between the types of antibody and complement may occur.

(6) Warm-active IgM occasionally fixes complement to C_9; severe intravascular hemolysis occurs with activation of membrane attack complex.

(7) Cold autoagglutinins that bind to the erythrocyte membrane below 10°C to 15°C are usually not significant and are observed in blood specimens from many healthy animals.

b. Decreased erythrocyte deformability

(1) Changes in the erythrocyte membrane, increase in internal viscosity, or decrease in surface area to volume ratio predisposes affected erythrocytes to early removal from circulation. These less deformable erythrocytes are sequestered in the spleen and phagocytosed by macrophages.

(2) Examples of decreased erythrocyte deformability (Figure 1.7)

(a) Schistocytes of microangiopathic anemia

(b) Spherocytes of immune-mediated anemia

(c) Parasitized erythrocytes

(d) Eccentrocytes or Heinz body-containing erythrocytes

Free ebooks ==> www.Ebook777.com

c. Reduced glycolysis and ATP content of the erythrocyte

(1) Affected erythrocytes are predisposed to removal from the vasculature by splenic macrophages.

(2) Reduction in glycolysis occurs with normal aging.

(3) This reduction is accelerated in hereditary pyruvate kinase and phosphofructokinase deficiency anemias.

d. Increased macrophage phagocytic activity

(1) When there is excessive macrophage phagocytic activity, normal erythrocytes also may be phagocytosed.

(2) Increased phagocytic activity is associated with conditions causing splenomegaly. Splenomegaly promotes excessive sequestration of erythrocytes and their exposure to macrophages.

(3) In humans, this condition has been called "hypersplenism."

(4) Increased macrophage phagocytic activity also occurs in hemophagocytic syndrome and malignant histiocytosis, resulting in cytopenias.

2. Clinical and laboratory characteristics of extravascular (phagocytic) hemolysis

a. The clinical course of disease is usually chronic with an insidious onset.

b. A regenerative response is associated with normal or increased plasma protein concentration.

c. Hemoglobinemia and hemoglobinuria are absent.

d. Hyperbilirubinemia occurs if the magnitude of the hemolysis is sufficient to exceed uptake, conjugation, and excretion of bilirubin by the liver. Unconjugated bilirubin usually predominates in early disease, but conjugated bilirubin may be prominent at a later time.

e. The bone marrow response may compensate for the destruction of erythrocytes in cases of low-grade hemolysis. In such instances, the Hct remains in the reference interval. This situation is referred to as a "compensated hemolytic anemia."

f. Neutrophilia, monocytosis, and thrombocytosis commonly accompany extravascular hemolysis.

g. Splenomegaly may result from increased macrophage activity and extramedullary hematopoiesis.

h. Low-grade extravascular hemolysis occurs in many anemias that are primarily nonhemolytic (e.g., anemia of chronic renal disease, iron-lack anemia). This is referred to as the "hemolytic component" of these other types of anemia.

3. Aids in identification of the specific cause of extravascular hemolysis

a. History of a particular breed and/or of affected littermates may suggest a hereditary cause of hemolysis. Examples include the following:

(1) Phosphofructokinase deficiency of American Cocker Spaniel, English Springer Spaniel, and mixed breed dogs with Spaniel heritage.

(2) Pyruvate kinase deficiency in dogs (Basenji, Beagle, Chihuahua, Dachshund, Pug, Miniature Poodle, West Highland White, American Eskimo Dog, and Cairn Terrier) and cats (Abyssinian, Somali, and domestic short haired).

(3) Possible hereditary predisposition to hemolytic anemia also may exist in other dog breeds (Border Collie, Cocker Spaniel, English Springer Spaniel, German Shepherd, Irish Setter, Old English Sheepdog, Poodle, and Whippet).

b. Additional laboratory findings

(1) Positive direct antiglobulin (Coombs') test against a specific immunoglobulin or C_3 on patient's erythrocytes in immune-mediated anemia or a positive DELAT test even if the direct antiglobulin test is negative.

(2) Abnormal erythrocyte morphology occurs in a variety of anemias (Figure 1.7). Erythrocytic parasites, spherocytes, schistocytes, and keratocytes suggest the potential for excessive phagocytosis of erythrocytes.

www.Ebook777.com

B. Intravascular hemolysis (Case 3). Erythrocytes are destroyed within the circulation, releasing hemoglobin into the plasma where it is either removed by the liver or excreted by the kidneys.

1. Mechanisms of intravascular hemolysis. The erythrocyte membrane must be significantly disrupted to allow escape of the Hb molecule into the plasma. Most of the mechanisms of intravascular hemolysis are extrinsic or extracorpuscular defects (i.e., the erythrocyte itself is initially normal).

a. Complement-mediated lysis

(1) Complement (C_3) is deposited onto the erythrocyte membrane by surface antigen-antibody reactions. If complement is activated to C_9, the membrane attack complex is formed, producing a large enough membrane defect for the escape of Hb.

(2) Complement-mediated lysis occurs most commonly in immune-mediated anemias when IgM is involved. IgM is very effective in fixing complement. In contrast, IgG fixes complement poorly but may occasionally cause complement-mediated lysis.

(3) IgM-mediated complement lysis is the mechanism in most cases of hemolytic disease of newborn foals (neonatal isoerythrolysis) and transfusion reactions (in cats and large animals). Occasionally, a similar mechanism involving IgM may occur in autoimmune hemolytic anemia.

(4) Complement fixation to C_3 promotes phagocytosis but not intravascular lysis.

b. Physical injury

(1) Traumatic disruption of the erythrocyte membrane can occur from the shearing effect of intravascular fibrin strands.

(2) Because fibrin usually is formed in small blood vessels, this type of anemia is called "microangiopathic anemia."

(3) Examples of microangiopathic anemia include disseminated intravascular coagulation, vasculitis, hemangiosarcoma, and heartworm disease.

(4) Schistocytes are fragmented erythrocytes whose membranes have been altered by trauma (Figure 1.7). Their presence suggests shearing of erythrocyte membranes and the presence of microangiopathic anemia.

c. Oxidative injury (Case 3)

(1) Oxidants affect the erythrocyte in three ways:

(a) Denaturation of Hb with Heinz body formation

(b) Oxidation and cross-linking of membrane proteins with eccentrocyte formation

(c) Oxidation of hemoglobin iron (Fe^{2+}) with the formation of methemoglobin (Fe^{3+}). This interferes with oxygen transport but does not cause anemia.

(2) Heinz body formation and oxidation of cell membranes can cause sufficient cellular damage for hemoglobin to escape from the cytoplasm.

(3) If intravascular lysis does not occur, these altered cells are removed prematurely from circulation by phagocytosis.

(4) The erythrocyte is protected from daily exposure to oxidants via two major pathways.

(a) Reduced glutathione, which neutralizes oxidants, is produced and maintained in the reduced state by the pentose phosphate (hexose monophosphate) pathway. Deficiency of enzymes and intermediates (e.g., glucose-6-phosphate dehydrogenase) in this pathway can lead to membrane oxidation and Heinz body formation in the presence of excessive exogenous oxidants.

(b) Iron is maintained in the reduced state by methemoglobin reductase; accumulation of methemoglobin is minimized. Methemoglobin reductase deficiency has been reported in dogs and one horse.

(5) In most cases, the offending oxidant is drug- or diet-derived. Either the oxidant or its intermediate metabolites directly oxidize or interfere with formation of reduced glutathione.

(6) Heinz bodies or eccentrocytes suggest oxidative damage (Figure 1.7).

d. Osmotic lysis

Free ebooks ==> www.Ebook777.com

(1) Hemolysis may be associated with hypophosphatemia, especially in patients with diabetes.
(2) Membrane alterations insufficient to allow leakage of Hb may alter permeability to the extent that excess water is drawn into the normally hypertonic cell and lysis occurs.
(3) Hypotonic intravenous fluids cause osmotic lysis.
(4) Cold hemoglobinuria of cattle is thought to occur by this mechanism.
(5) Many of the causes listed under extravascular hemolysis (Table 1.2) may alter the membrane of some cells to the degree that osmotic lysis occurs prior to phagocytosis.

e. Membrane alterations by other mechanisms
(1) Castor beans contain ricin, which causes direct membrane lysis.
(2) Snake venoms have lytic properties.
(3) Bacterial toxins, such as the phospholipase of *Clostridium novyi*, directly attack membrane lipids.
(4) *Babesia* spp. multiply in the erythrocyte and disrupt the membrane upon exiting the cell.

f. Intravascular causes of hemolysis do not lyse all affected erythrocytes; some altered cells may remain in circulation and are subsequently removed by phagocytosis.

2. Clinical and laboratory characteristics of intravascular hemolytic anemia
a. Intravascular hemolysis usually presents as a peracute or acute disease.
b. History may reveal exposure to causative drugs or plants, recent transfusion of incompatible blood, or recent ingestion of colostrum.
c. A regenerative response occurs, but it may not be evident in early stages because two to three days are required before significant reticulocytosis occurs.
d. Hemoglobinemia (free Hb in the plasma) is the principal feature of intravascular hemolysis. Hemoglobinemia is usually detected by the following:
(1) Red discoloration of plasma
(2) Increased MCHC and MCH
(3) Decreased serum haptoglobin and hemopexin concentrations
e. Hemoglobinuria may occur 12 to 24 hours following hemolysis if the concentration of free hemoglobin saturates the available haptoglobin and hemopexin and exceeds the capacity of the renal tubular epithelial cells to absorb and metabolize any hemoglobin that passes the glomerular filter.
f. Hemosiderinuria occurs if there is sufficient renal tubular epithelial cell absorption and metabolism to form detectable hemosiderin.
g. Hyperbilirubinemia
(1) Bilirubin is not formed until 8 to 10 hours after the onset of the hemolytic episode.
(2) Hyperbilirubinemia will occur if bilirubin formation is of sufficient magnitude to exceed the capacity of the liver to remove bilirubin from plasma, conjugate it, and excrete it into bile.
(3) Unconjugated bilirubin is the predominant form early in the disease. Conjugated bilirubin becomes more prominent with time and occasionally may be the major form present. Conjugated hyperbilirubinemia is accompanied by bilirubinuria (Chapters 7 and 9).
h. Additional laboratory findings may include schistocytes, keratocytes, Heinz bodies, eccentrocytes, erythrocytic parasites (Figure 1.7), positive direct antiglobulin (Coombs') test on patient's erythrocytes, or antibody titer to or culture of potentially causative organisms.

ANEMIA FROM REDUCED OR DEFECTIVE ERYTHROPOIESIS (TABLE 1.3)

Anemias caused by reduced or defective erythropoiesis are nonregenerative. They are characterized by an abnormal bone marrow that cannot maintain effective erythropoiesis. The clinical course is usually long

www.Ebook777.com

TABLE 1.3.
CAUSES OF REDUCED OR DEFECTIVE ERYTHROPOIESIS.

Reduced erythropoiesis	Defective erythropoiesis
Anemia of chronic disorders	Abnormal maturation
Chronic inflammation	Congenital dyserythropoiesis of Herefords
Neoplasia	Dyserythropoiesis of English Springer Spaniels
Cytotoxic bone marrow damage	Erythremic myelosis
Bracken fern	Erythroleukemia
Cytotoxic cancer drugs	Macrocytosis of Poodles
Estrogen	Myelodysplastic syndrome
Furazolidone	Disorders of heme synthesis
Phenylbutazone	Chloramphenicol toxicity
Radiation	Copper deficiency
Erythropoietin lack	Iron deficiency
Chronic renal disease	Lead poisoning
Hypoadrenocorticism	Molybdenum poisoning
Hypoandrogenism	Pyridoxine deficiency
Hypopituitarism	Disorders of nucleic acid synthesis
Hypothyroidism	B_{12} deficiency, malabsorption
Immune-mediated	Folic acid deficiency
Pure red cell aplasia	
Infections	
Anaplasma (formerly *Ehrlichia*) spp.	
Feline leukemia virus	
Feline panleukopenia virus	
Parvovirus	
Trichostrongyles (non-bloodsucking)	
Myelophthisis	
Lymphocytic leukemia	
Metastatic neoplasia	
Myelofibrosis	
Myeloproliferative disorders	
Osteopetrosis, osteosclerosis	

and the onset insidious. Nonregenerative anemias, such as the anemia of inflammatory disease, are observed commonly in veterinary medicine.

I. GENERAL CONSIDERATIONS

A. Mechanisms

1. Ability of the bone marrow to sustain erythrocytic mass requires the following items to be adequate:

a. Precursor cells (i.e., multi- and unipotential stem cells)

b. Nutrients (e.g., iron and B vitamins)

c. Stimulation (e.g., Epo, IL-3, G-CSF, GM-CSF)

d. Microenvironment

2. Primary and secondary bone marrow failure

Free ebooks ==> www.Ebook777.com

a. Primary bone marrow failure from intramarrow disease results in inadequate production of stem and progenitor cells.

b. Secondary bone marrow failure occurs from extramarrow causes such as lack of nutrients or growth factors (Epo, colony stimulating factors, or cytokines).

3. Bone marrow failure may be selective for the erythroid series (e.g., pure red cell aplasia) or may affect granulocytes and/or platelet-producing megakaryocytes leading to bicytopenia or pancytopenia. In pancytopenia (aplastic anemia), a nonregenerative anemia occurs concurrently with granulocytopenia and thrombocytopenia.

B. Bone marrow response

1. When the number of precursor cells or erythropoietic stimulation is inadequate, the erythroid marrow is normo- to hypocellular.

2. Maturation abnormalities are associated with a hypercellular marrow and ineffective erythropoiesis (i.e., failure of the erythrocytes produced to mature normally or to be delivered to the blood). Maturation abnormalities are especially prominent in nutritional deficiencies, myelodysplastic syndrome (MDS), leukemias, and hereditary dyserythropoiesis. Abnormal cells such as microcytes, macrocytes, or nRBCs usually are observed in the stained blood film.

3. All degrees of bone marrow failure can occur, from suboptimal response of the erythroid marrow following hemorrhage or hemolysis to complete aplasia.

II. DIFFERENTIATION OF ANEMIAS CAUSED BY REDUCED OR DEFECTIVE ERYTHROPOIESIS

This practical approach to the diagnosis of nonregenerative anemias is based on erythrocyte morphology, blood neutrophil and platelet numbers, and bone marrow cellularity. These anemias can be divided into the following hematologic patterns.

A. Normocytic, normochromic anemia with normal to increased neutrophil and platelet counts, and an increased M:E ratio caused by hypocellular erythroid marrow (this is expected but not always readily apparent). These general types of anemia include the following:

1. Anemia of erythropoietin lack caused by certain diseases:

a. Chronic renal disease (Cases 15, 19)

(1) The degree of anemia is roughly proportional to the severity of the uremia.

(2) Causes for the development of anemia

(a) Erythropoietin deficiency caused by destruction of the Epo-secreting peritubular interstitial cells

(b) Hemolysis caused by factors in uremic plasma

(c) Gastrointestinal hemorrhage from abnormal platelet function and vascular lesions

(d) Inhibitors of erythropoiesis in uremic plasma

b. Endocrinopathies (e.g., hypoadrenocorticism, hypoandrogenism, and hypopituitarism)

(1) Some of these hormones (e.g., androgens) may enhance the action of erythropoietin.

(2) In other cases, the exact mechanism of anemia is unknown.

2. Anemia of inflammatory disease (AID) (Cases 7, 8, 10, 11)

a. AID (anemia of chronic disease [ACD], anemia of chronic disorders) occurs in chronic infectious, inflammatory, or neoplastic disorders. The onset of anemia may be as short as three to 10 days.

b. AID is mediated by hepcidin, a peptide made within and released by the liver in response to inflammation and induced by the proinflammatory cytokine interleukin-6.

c. Diminished marrow responsiveness to erythropoietin, blunted erythropoietin release, and impaired availability of iron to the erythron are all involved in the pathogenesis of anemia.

d. Erythrocyte life span is shortened.

e. Laboratory findings in AID:

www.Ebook777.com

(1) Decreased to normal serum iron concentration
(2) Decreased to normal total iron-binding capacity
(3) Normal to increased serum ferritin concentration
(4) Normal to increased bone marrow macrophage iron stores
(5) Mild to moderate anemia (Hct 20% to 30%) that is usually nonprogressive
(6) Normocytic, normochromic erythrocyte indices
(7) Microcytosis and hypochromia rarely occur. Sequestration of iron must be markedly prolonged to cause microcytosis and hypochromia in AID.
(8) Increased serum copper and zinc concentrations
(9) Variable serum erythropoietin concentration
(10) Increased serum hepcidin concentration

f. Signs of inflammatory or neoplastic disease often dominate the clinical picture, obscuring signs of anemia.

g. If the primary disease process is alleviated, then recovery from AID follows.

3. Feline leukemia virus (FeLV)-associated nonregenerative anemia

a. Erythroid stem and progenitor cells are selectively killed by FeLV.

b. The anemia may be macrocytic due to asynchronous maturation.

4. Pure red cell aplasia (PRCA)

a. Anemia is characterized by a selective loss of erythroid precursors in the bone marrow.

b. PRCA appears to be immune-mediated because it may respond to treatment with corticosteroids and/or lymphocytotoxic drugs.

c. Some cases of PRCA have been positive by Coombs' testing and have been designated as "nonregenerative autoimmune hemolytic anemia."

d. Rarely, PRCA may be observed secondary to lymphoma.

5. Unknown mechanisms of nonregenerative anemia

a. Trichostrongyle infection (non-blood sucking) in cattle and sheep

b. Liver disease

c. Vitamin E deficiency

(1) Dietary deficiency of vitamin E may produce a nonregenerative anemia in swine.
(2) The erythroid marrow is hyperplastic with evidence of dyserythropoiesis.

B. Normocytic, normochromic anemia with neutropenia (except in the case of myeloproliferative disorders) and/or thrombocytopenia, variable M:E ratio. Generalized bone marrow hypocellularity and/or proliferation of abnormal cells may be present. These types of anemia include the following:

1. Aplastic anemia or pancytopenia (Case 4)

a. This is a disease of the multipotential stem cell or bone marrow microenvironment that leads to pancytopenia and an acellular, fatty bone marrow.

b. Concomitant deficiencies in erythropoiesis, granulopoiesis, and thrombopoiesis occur. Leukopenia and thrombocytopenia usually precede the development of anemia because of the shorter life span of leukocytes and platelets.

c. Causes of aplastic anemia

(1) Predictable or idiosyncratic drug reactions that can vary by species (e.g., late estrogen toxicosis in dogs, chloramphenicol toxicosis in cats, phenylbutazone, trimethoprim-sulfadiazine, albendazole)
(2) Chemical exposure and plant toxicosis (e.g., bracken fern in ruminants and horses)
(3) Irradiation
(4) Cytotoxic T-cells or antibody (suggested to be involved in some human aplastic anemias)
(5) Infectious agents (e.g., FeLV in cats, ehrlichiosis in dogs)

2. Myelophthisic anemia

a. In myelophthisic anemia, the bone marrow is physically replaced by an abnormal proliferation of stromal, inflammatory, or neoplastic cells. Examples of disease leading to myelophthisic anemia:

Free ebooks ==> www.Ebook777.com

(1) Myeloproliferative disorders (e.g., hematopoietic malignancies, leukemias)
(2) Myelofibrosis
(3) Osteosclerosis
(4) Diffuse granulomatous osteomyelitis
(5) Metastatic cancer

b. Bone marrow aspirates may yield very few cells in some conditions (e.g., myelofibrosis) and many cells in other instances (myeloproliferative disorders). Core biopsies are preferred to diagnose stromal reactions.

c. Leukoerythroblastic reactions (metarubricytosis without reticulocytosis and a neutrophilic left shift in the absence of inflammation) may occur from disruption of marrow architecture with disorderly release of myeloid and erythroid precursor cells.

d. A mild regenerative response, caused by isolated erythroid foci, may be observed in the early stages of myelophthisic anemia.

e. Dacryocytes (teardrop-shaped poikilocytes) are present in human blood smears, but have not been recognized consistently in animal blood smears.

f. Myelophthisic anemia caused by myeloproliferative disorders often have a leukocytosis with a leukemic blood picture.

3. Nonregenerative anemias caused by infectious agents

a. Acute ehrlichiosis may present as pancytopenia. In chronic ehrlichiosis, only mild thrombocytopenia may be evident on the hemogram.

b. FeLV infection occasionally may cause concomitant anemia and leukopenia, resulting in pancytopenia. FeLV-induced anemias may be macrocytic.

c. Feline and canine parvoviral infections destroy hematopoietic cells, lymphoid cells, and gastrointestinal crypt epithelial cells. Neutropenia is often evident on the leukogram. Despite the destruction of hematopoietic cells by parvovirus, the anemia is usually masked by relative polycythemia from dehydration secondary to vomiting, diarrhea, and lack of fluid intake.

d. Canine parvoviral infection of sufficient duration can produce concurrent neutropenia and nonregenerative anemia.

C. Microcytic, hypochromic anemia with variable neutrophil and platelet counts, and usually a hypercellular marrow with a variable M:E ratio. Causes of this type of anemia include the following:

1. Iron deficiency (Case 5)

a. The most common cause of iron deficiency is chronic hemorrhage, usually with external blood loss.

b. Young, rapidly growing animals consuming an all-milk diet may have transient dietary iron deficiency resulting in mild anemia.

c. Iron deficiency anemia is associated with ineffective erythropoiesis and a hyperplastic marrow early in the disease process. With chronicity, the bone marrow becomes hypoplastic and both microcytosis and hypochromia are more evident.

d. Laboratory findings in iron deficiency anemia

(1) Decreased serum iron concentration
(2) Variable total iron-binding capacity, but TIBC is often within the reference interval or increased
(3) Low percent saturation of transferrin
(4) Decrease or absence of marrow macrophage iron stores
(5) Decreased serum ferritin concentration
(6) Increased free erythrocyte protoporphyrin
(7) Microcytosis. Microcytosis develops because the critical concentration of hemoglobin necessary to stop cell division is not reached, and an extra division occurs. Microcytosis often precedes hypochromasia.
(8) Hypochromasia is present in most species, but may not be observed in the cat.

www.Ebook777.com

(9) Poikilocytes (e.g., schistocytes, keratocytes). Poikilocytes are thought to result from oxidation of membrane proteins.
(10) Hypercellular bone marrow in early disease with a disproportionate number of late rubricytes and metarubricytes due to extra cell divisions.
(11) Low serum Hepcidin concentration.

2. Pyridoxine deficiency. This vitamin is a cofactor in heme synthesis. Pyridoxine deficiency leads to a failure to utilize iron and an iron-lack-type anemia.
3. Copper deficiency. Copper-containing ceruloplasmin and hephaestin are important in iron absorption and transfer between intestine, macrophages, and transferrin. Therefore, copper deficiency leads to iron deficiency.
4. Dyserythropoiesis in English Springer Spaniels. This disease is associated with polymyopathy and cardiac disease and is characterized by a microcytic, nonregenerative anemia with metarubricytosis and dysplastic change of nucleated erythroid cells.
5. Microcytosis without anemia occurs in the Asian dog breeds including the Akita, Chow Chow, Shar Pei, and Shiba Inu. Microcytosis also has been reported in a dog with hereditary elliptocytosis.
6. Microcytosis with mild anemia is a common finding in portosystemic shunts (PSS) in the dog (Case 13). Alterations in iron metabolism appear similar to those in the anemia of inflammatory disease. Normal to decreased serum iron concentration and serum TIBC occur in nearly half of affected dogs. Serum ferritin concentrations are normal to increased in conjunction with stainable marrow and hepatic iron stores. One-third of cats with PSS exhibit microcytosis, but anemia usually is not observed.
7. Members of the Camelidae family, including llamas, have elliptical erythrocytes. In iron deficiency, these erythrocytes are microcytic and exhibit irregular or eccentric areas of hypochromasia representing irregular Hb distribution within the cell.
8. Drugs or chemicals, including chloramphenicol and lead toxicoses, can block synthesis of heme.

D. Macrocytic, normochromic anemia with variable neutrophil and platelet counts. M:E ratio is usually low because of hypercellular erythroid marrow. Causes of macrocytic, normochromic anemia include the following:

1. Ruminants grazing cobalt-deficient or molybdenum-rich pastures
2. Vitamin B_{12} and folic acid deficiencies
a. This type of anemia has not been produced experimentally in animals, but macrocytic anemias that responded to these vitamins have been described (e.g., Giant Schnauzers).
b. Megaloblastoid erythroid precursors are observed in the bone marrow.
c. Enlarged, hypersegmented neutrophils may be observed in the blood smear.
d. The hypercellular bone marrow indicates ineffective erythropoiesis.
3. Erythremic myelosis or erythroleukemia (Chapter 3)
4. Congenital dyserythropoiesis and progressive alopecia of polled Hereford calves. This disease is characterized by a macrocytic, nonregenerative anemia with ineffective erythropoiesis.
5. FeLV infection. Cats may present with a macrocytic anemia, but the erythroid marrow is usually hypocellular.
6. Macrocytosis of Poodles. This hereditary condition is uncommon. Neither anemia nor reticulocytosis occurs. Erythrocyte counts typically are within the low end of the reference interval and MCVs often are very high (over 100 fL).

POLYCYTHEMIA

Polycythemia is an increase in the Hct, RBC count, and Hb concentration.

Free ebooks ==> www.Ebook777.com

I. SPURIOUS OR RELATIVE POLYCYTHEMIA. THE TOTAL RBC MASS IS NORMAL. CAUSES OF RELATIVE POLYCYTHEMIA INCLUDE THE FOLLOWING:

A. Dehydration (Cases 6, 9, 18, 24)

1. A decrease in plasma volume causes a relative increase in the Hct, RBC count, Hb concentration, and plasma protein concentration.

2. Determination of dehydration is based on physical examination and not on laboratory tests.

3. Mechanisms of relative polycythemia

a. Water loss caused by vomiting, diarrhea, excessive diuresis, water deprivation, perspiration, or febrile dehydration

b. Internal fluid loss in shock via increased vascular permeability

c. Loss of fluid by effusion into body cavities

4. The Hct of sick animals may fluctuate 2% to 5% daily as a consequence of changes in the patient's hydration status.

B. Redistribution of erythrocytes

1. Excitement causes epinephrine release and splenic contraction. Splenic contraction delivers high-Hct splenic blood (Hct = 80%) into the general circulation.

2. This effect is common in the horse and cat.

II. ABSOLUTE POLYCYTHEMIA

Increased erythropoiesis expands the total RBC mass. Plasma volume and plasma protein concentration are within the reference interval.

A. Primary absolute polycythemia (polycythemia vera or primary erythrocytosis) is a myeloproliferative disorder of stem cells.

1. Clinical pathology findings include the following:

a. Erythropoietin concentration is within the reference interval or decreased.

b. PO_2 is within the reference interval.

c. Thrombocytosis and leukocytosis occasionally accompany the erythrocytosis.

B. Secondary absolute polycythemia is caused by increased Epo secretion.

1. Appropriate, compensatory Epo secretion occurs during chronic hypoxia (low PO_2) which occurs in the following instances:

a. High altitude

b. Chronic pulmonary disease

c. Cardiovascular anomalies with right to left shunting of blood

2. Inappropriate Epo secretion (normal PO_2, no hypoxia) occurs in some cases of hydronephrosis or renal cysts, Epo-secreting neoplasms (embryonal nephroma, renal carcinoma, uterine leiomyoma, cerebellar hemangioma, hepatoma, other endocrine neoplasms), and certain endocrinopathies (Case 27).

C. Animals with severe polycythemia may present for seizures because of blood sludging and CNS ischemia.

REFERENCES

Abboud CN, Lichtman MA: 1995. Structure of the bone marrow. *In:* Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds): *Williams Hematology*, 5th ed. McGraw-Hill, Inc., New York, pp. 25–38.

www.Ebook777.com

Barker RN, Gruffydd-Jones TJ, Stokes CR, et al: 1992. Autoimmune haemolysis in the dog: Relationship between anemia and the levels of red blood cell bound immunoglobulins and complement measured by an enzyme-linked antiglobulin test. *Vet Immunol Immunopathol* 34:1–20.

Campbell KL: 1990. Diagnosis and management of polycythemia in dogs. *Compend Contin Educ Pract Vet* 12:543–550.

Car BD: 2000. Erythropoiesis and erythrokinetics. *In*: Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Philadelphia, Lippincott Williams and Wilkins, pp. 105–109.

Carney HC, England JJ: 1993. Feline hemobartonellosis. *Vet Clin North Am Small Anim Pract* 23:79–90.

Christopher MM, Lee SE: 1994. Red cell morphologic alterations in cats with hepatic disease. *Vet Clin Pathol* 23:7–12.

Cook SM, Lothrop CD: 1994. Serum erythropoietin concentrations measured by radioimmunoassay in normal, polycythemic, and anemic dogs and cats. *J Vet Intern Med* 8:18–25.

Day MJ: 2000. Immune-mediated hemolytic anemia. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Baltimore, pp. 799–806.

Desnoyers M: 2000. Anemias associated with Heinz bodies. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Baltimore, pp. 178–184.

Edwards CJ, Fuller J: 1996. Oxidative stress in erythrocytes. *Comp Haematol Int* 6:24–31.

Franco DA, Lin TL, Leder JA: 1992. Bovine congenital erythropoietic porphyria. *Compend Contin Educ Pract Vet* 14:822–825.

Fyfe JC, Giger U, Hall CA, et al: 1991. Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs. *Pediatr Res* 29:24–31.

Gaunt SD: 2000. Hemolytic anemias caused by blood rickettsial agents and protozoa. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Philadelphia, Lippincott Williams and Wilkins, pp. 154–162.

Giger U: 1992. Erythropoietin and its clinical use. *Compend Contin Educ Prac Vet* 14:25–34.

Giger U: 2000. Erythrocyte phosphofructokinase and pyruvate kinase deficiencies. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Baltimore, pp. 1020–1025.

Giger U: 2000. Regenerative anemia caused by blood loss or hemolysis. *In:* Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat*, 5th ed., Vol. 2. Philadelphia, WB Saunders Co, pp. 1784–1804.

Giger U, Kiltrain CG, Filippich LJ, Bell K: 1989. Frequencies of feline blood groups in the United States. *J Am Vet Med Assoc* 195:1230–1232.

Hagemoser WA: 1993. Comments on reticulocyte lifespans. *Vet Clin Pathol* 22:4–5.

Harvey JW: 1997. The erythrocyte: Physiology, metabolism, and biochemical disorders. *In: Clinical Biochemistry of Domestic Animals*, 5th ed. San Diego, Academic Press, pp. 157–203.

Harvey JW: 2000. Hereditary methemoglobinemia. *In* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Philadelphia, Lippincott Williams and Wilkins, pp. 1008–1011.

Holland CT, Canfield PJ, Watson ADJ, et al: 1991. Dyserythropoiesis, polymyopathy, and cardiac disease in three related English springer spaniels. *J Vet Intern Mod* 5:151–159.

Jain NC: 1993. *Essentials of Veterinary Hematology*. Philadelphia, Lea and Febiger.

Free ebooks ==> www.Ebook777.com

Kazmierski KJ, Ogilvie GK, Fettman MJ, et al: 2001. Serum zinc, chromium, and iron concentrations in dogs with lymphoma and osteosarcoma. *J Vet Intern Med* 15:585–588.

Kemna E, Tjalsma H, Willems H, et al: 2008. Hepcidin: from discovery to differential diagnosis. *Haematologica* 93:90–97.

King LG, Giger U, Diserens D, et al: 1992. Anemia of chronic renal failure in dogs. *J Vet Intern Med* 6:264–270.

Klag AR, Giger U, Shofer FS: 1993. Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986–1990). *J Am Vet Med Assoc* 202:783–788.

Kociba GJ: 2000. Macrocytosis. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Philadelphia, Lippincott Williams and Wilkins, pp. 196–199.

McConnico RS, Roberts MC, Tompkins M: 1992. Penicillin-induced immune-mediated hemolytic anemia in a horse. *J Am Vet Med Assoc* 201:1402–1403.

Mair TS, Taylor EGR, Hillyer MH: 1990. Autoimmune haemolytic anaemia in eight horses. *Vet Rec* 126:51–53.

Means RT, Krantz SB: 1992. Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood* 80:1639–1647.

Meyer DJ, Harvey JW: 1994. Hematologic changes associated with serum and hepatic iron alterations in dogs with congenital portosystemic vascular anomalies. *J Vet Intern Med* 8:55–56.

Morgan RV, Moore FM, Pearce LK, et al: 1991.Clinical and laboratory findings in small companion animals with lead poisoning: 347 cases (1977–1986). *J Am Vet Med Assoc* 1909:93–97.

Morin DE, Garry FB, Weiser MG, et al: 1992. Hematologic features of iron deficiency anemia in llamas. *Vet Pathol* 29:400–404.

Ogawa E, Koboyaski K, Yoshiura N, et al: 1987. Bovine postparturient hemoglobinemia: Hypophosphatemia and metabolic disorder in red blood cells. *Am J Vet Res* 48:13001303.

Penedo MCT: 2000. Red blood cell antigens and blood groups in the cow, pig, sheep, goat and llama. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Baltimore, pp. 778–782.

Rebar AH, Lewis HB, DeNicola DB, et al: 1981. Red cell fragmentation in the dog: An editorial review. *Vet Pathol* 18:415–426.

Rottaman JB, English RV, Breitschwerdt EB, et al: 1991. Bone marrow hypoplasia in a cat treated with griseofulvin. *J Am Vet Med Assoc* 354:429–431.

Smith JE: 1992. Iron metabolism in dogs and cats. *Compend Contin Educ Pract Vet* 14:39–43.

Steffen DJ, Elliott GS, Leipold HW, et al: 1992. Congenital dyserythropoiesis and progressive alopecia in polled Hereford calves: Hematologic, biochemical, bone marrow cytology, electrophoretic, and flow cytometric findings. *J Vet Diagn Invest* 4:31–37.

Stone MS, Freden GO: 1990. Differentiation of anemia of inflammatory disease from anemia of iron deficiency. *Compend Contin Educ Pract Vet* 12:963–966.

Swenson CL, Kociba GJ, O'Keefe DA, et al: 1987. Cyclic hematopoiesis associated with feline leukemia virus infection in two cats. *J Am Vet Med Assoc* 191:93–96.

Tvedten H, Weiss D: 1999. The complete blood count and bone marrow examination: General comments and selected techniques. *In:* Willard MD, Tvedten H, Turnwald GH (eds): *Small Animal Clinical Diagnosis by Laboratory Methods*, 3rd ed. Philadelphia, W. B. Saunders, pp. 11–30.

www.Ebook777.com

Tvedten H, Weiss DJ: 2000. Classification and laboratory evaluation of anemia. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Philadelphia, Lippincott Williams and Wilkins, pp. 143–150.

Weiss DJ: 1984. Uniform evaluation and semiquantitative reporting of hematologic data in veterinary laboratories. *Vet Clin Pathol* 13:27–31.

Weiss DJ, Geor RJ: 1993. Clinical and rheological implications of echinocytosis in the horse: a review. *Comp Haematol Int* 3:185–189.

Weiss DJ, Klausner JS: 1990. Drug-associated aplastic anemia in dogs: Eight cases (1984–1988). *J Am Vet Med Assoc* 196:472–475.

Free ebooks ==> www.Ebook777.com

CHAPTER 2

LEUKOCYTES

Julie L. Webb, DVM, and Kenneth S. Latimer, DVM, PhD

ESSENTIAL CONCEPTS OF LEUKOCYTE MORPHOLOGY, FUNCTION, PRODUCTION, AND KINETICS

Mammalian leukocytes include the neutrophil, monocyte, eosinophil, basophil, and lymphocyte. Heterophils are the avian equivalent of mammalian neutrophils. All leukocytes participate in body defense, but each is functionally independent. A general outline of hematopoiesis, including leukocytes, is presented in Figure 1.2 in Chapter 1.

I. NEUTROPHILS AND AVIAN HETEROPHILS

A. Morphology (Figure 2.1)

1. Mature neutrophils of mammals have multiple nuclear lobes separated by constrictions (polymorphonuclear) and have colorless to pale pink cytoplasm. Avian heterophils have fewer nuclear lobes but prominent dull red cytoplasmic granulation.

2. Granules are of two types.

a. Primary or azurophilic granules

(1) Primary granules stain reddish-purple with Romanowsky stains but generally do not stain and are not visible after the promyelocyte stage of development.

(2) Formation ceases early and they subsequently are diluted by cellular division.

(3) They are lysosomes and contain microbiocidal elements (myeloperoxidase, lysozyme, defensins, and bacterial-permeability-inducing protein) and enzymes (acid hydrolases, neutral proteases, napthol AS-D choloracetate esterase, and elastase).

(4) Avian heterophils lack significant myeloperoxidase activity.

(5) Ultrastructurally, primary granules are larger and more electron dense than secondary granules.

b. Secondary or specific granules

(1) Neutrophil secondary granules usually are not visible with Romanowsky stains.

(2) Secondary granules of avian heterophils appear dull red and elongate with pointed ends.

(3) Granule contents include microbiocidal elements (lactoferrin, lysozyme, cathelicidins) and enzymes (collagenase, apolactoferrin plasminogen activator).

(4) Alkaline phosphatase (ALP) activity is present in neutrophils of healthy cattle and horses; neutrophils of other domestic mammals and birds lack ALP activity.

(5) Ultrastructurally, secondary granules are more electron lucent than primary granules.

3. The stages of neutrophil maturation (Figure 2.2; also see Figure 1.7 in Chapter 1)

www.Ebook777.com

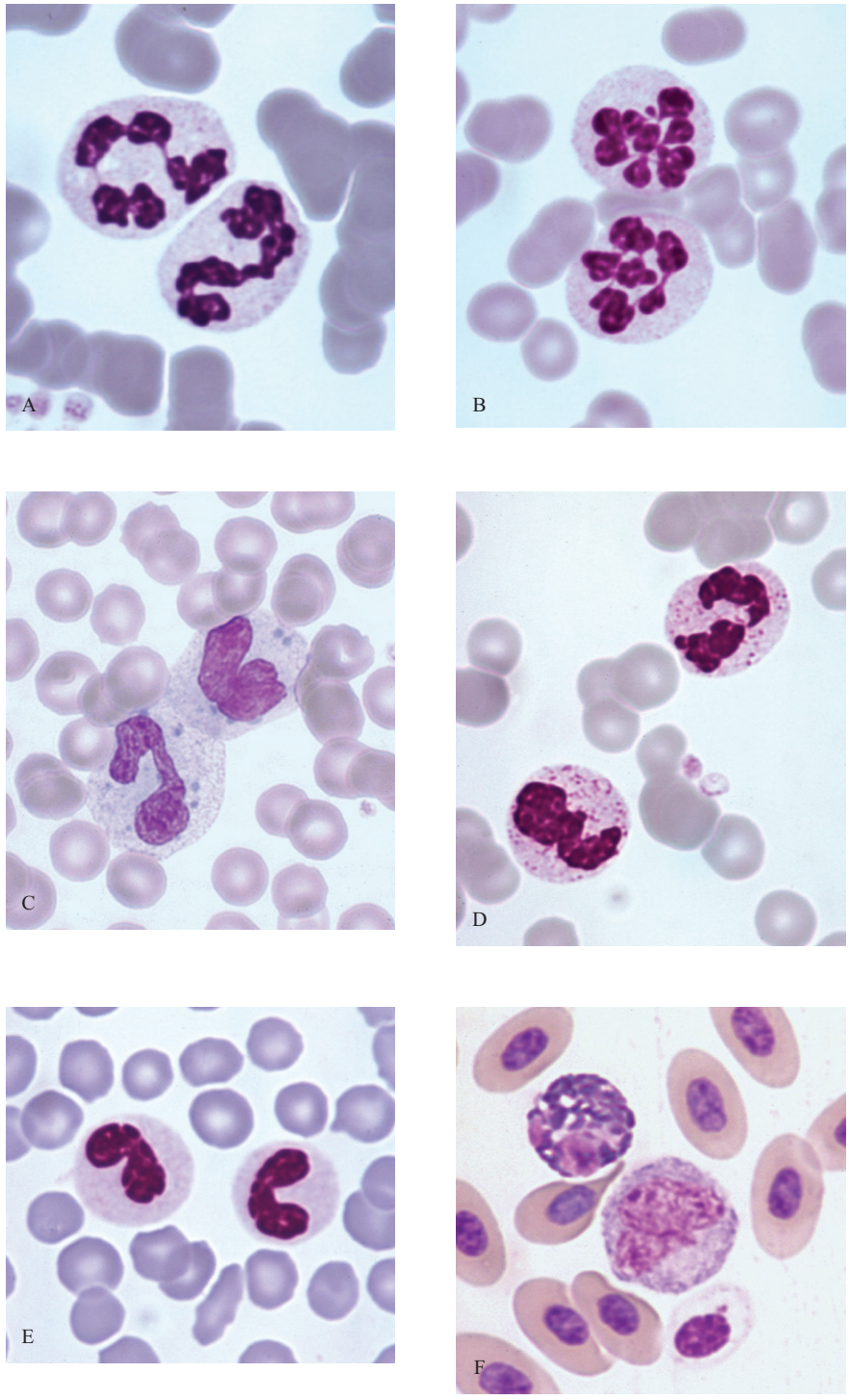
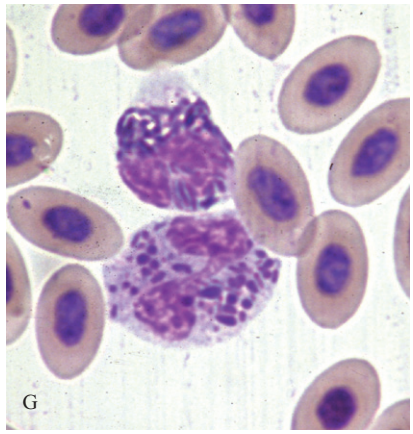
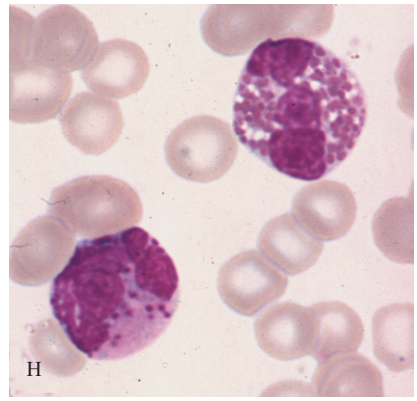
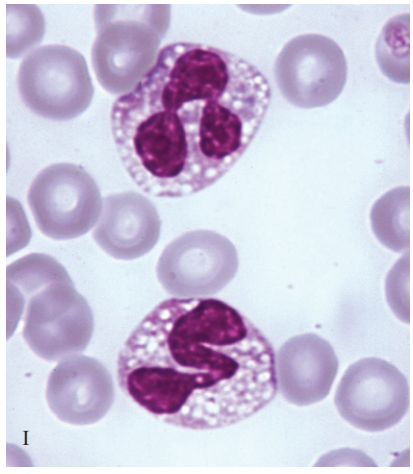


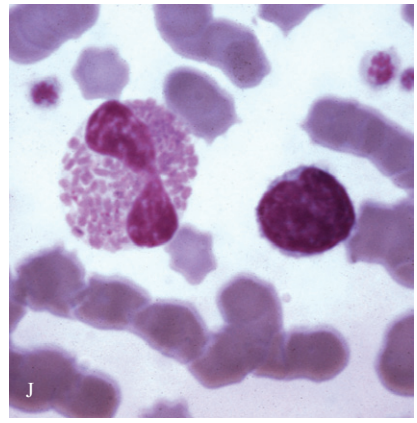
FIGURE 2.1. Leukocyte morphology in health and disease. A. Pair of segmented equine neutrophils; B. equine neutrophils with nuclear hypersegmentation; C. canine neutrophils with toxic change (cytoplasmic basophilia, vacuolation, and Döhle bodies); D. equine neutrophils with toxic granulation; E. neutrophils from a cat with Pelger-Huët anomaly; F. avian heterophil, monocyte, and thrombocyte; continued.

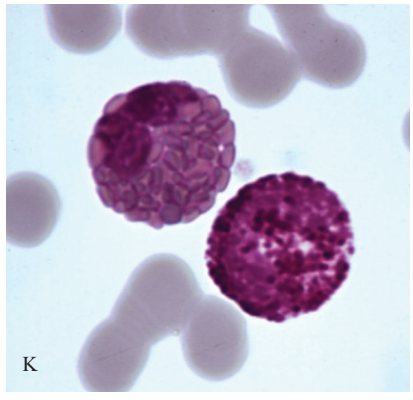
Free ebooks ==> www.Ebook777.com











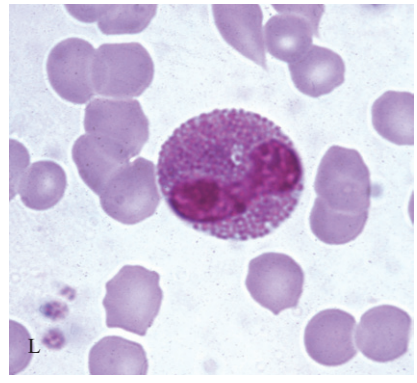
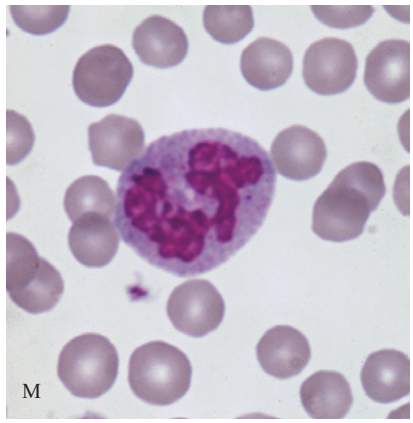
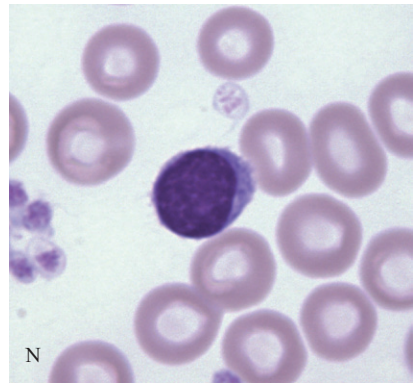
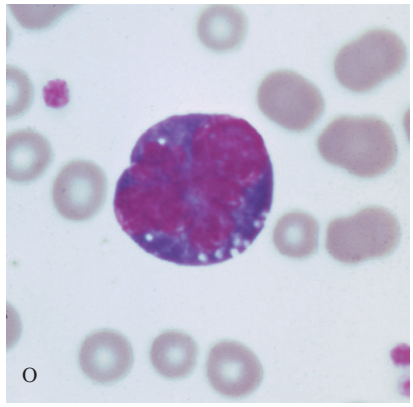


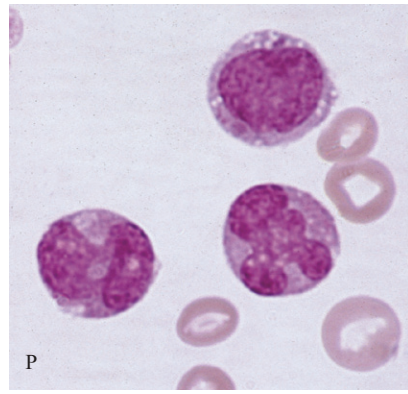
FIGURE 2.1. continued. G. normal and toxic avian heterophils; H. canine eosinophil and basophil; I. pair of degranulated canine eosinophils; J. feline basophil and small lymphocyte; K. equine eosinophil and basophil; L. porcine eosinophil; continued.

www.Ebook777.com









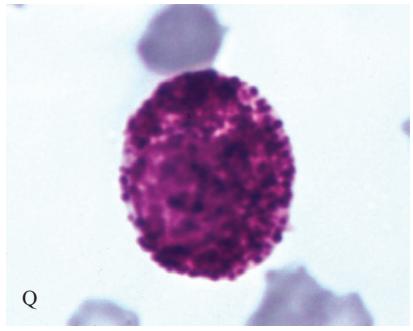


FIGURE 2.1. continued. M. feline basophil; N. canine mature small lymphocyte; O. canine reactive lymphocyte or immunocyte; P. three canine monocytes; Q. mast cell in the blood of a dog with enteritis.

a. Myeloblast
  (1) The nucleus is round to oval with dispersed chromatin and nucleoli.
  (2) The type I myeloblast lacks visible granules; the type II myeloblast may have a few (less than 15) primary (azurophilic) cytoplasmic granules.

b. Promyelocyte (progranulocyte)
  (1) The nucleus is round to oval with dispersed chromatin; nucleoli or nucleolar rings may be present.

Free ebooks ==> www.Ebook777.com

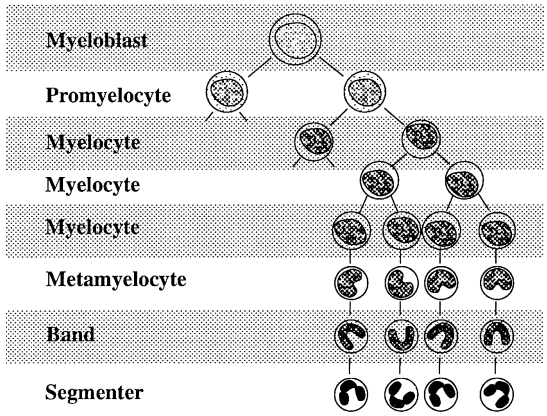


FIGURE 2.2. Sequence of granulopoiesis.

(2) Many primary (azurophilic), cytoplasmic granules characterize this stage of development.

c. Myelocyte

(1) The nucleus is oval with some aggregation of chromatin; nucleoli are not visible.

(2) Primary (azurophilic) granules lose their staining characteristics in this and subsequent stages of maturation and are not visible.

d. Metamyelocyte

(1) The nucleus is thick and indented with bulbous ends.

(2) Chromatin is more aggregated than in previous stages of development.

e. Band

(1) The nucleus is U- or S-shaped and of uniform thickness, but not as thick as that of metamyelocytes.

(2) Chromatin is more aggregated than that of metamyelocytes.

f. Segmenter

(1) The nucleus is separated into two to five lobes by constrictions. Filaments between nuclear segments, which are common in human neutrophils, are rare in neutrophils and heterophils of animals. The chromatin is very densely aggregated.

(2) Equine segmented neutrophils may have sharp, jagged nuclear membranes and indistinct nuclear lobes.

(3) The cytoplasm of bovine neutrophils is orange to pink with Romanowsky stains. The cytoplasm of other mammalian neutrophils usually is colorless, but may occasionally have very faint pink granulation.

(4) Avian heterophils have two to three nuclear lobes and dull-red, needle-shaped, cytoplasmic granules.

B. Regulation of granulopoeisis

1. During microbial invasion or other tissue injury that produces a demand for neutrophils, T lymphocytes, macrophages, and stromal cells are activated and release growth factors, including the following:

a. Colony-stimulating factors (CSFs) such as stem cell factor (SCF), granulocyte-CSF (G-CSF), and granulocyte/macrophage-CSF (GM-CSF).

b. Cytokines, including lymphokines and interleukins (IL-3, IL-6, and IL-11), stimulate endothelial cells and fibroblasts to produce CSFs.

2. CSFs are glycoproteins that act directly on hematopoietic subpopulations in the bone marrow, which results in the following:

a. Increased cellular proliferation

www.Ebook777.com

b. Cellular differentiation
c. Inducement of and enhanced cell function

3. Functions of other mediators of the inflammatory response
a. Stimulating bone marrow release of neutrophils
b. Promoting margination and adhesion of neutrophils to endothelium in affected tissues
c. Stimulating emigration of neutrophils through the endothelium at the site of inflammation
d. Inducing chemotaxis toward the site of tissue injury
e. Enhancing phagocytosis and microbicidal activity

4. The end result is an increase in the number of functioning neutrophils at the site of tissue injury.

C. Production

1. Neutrophil production and maturation take place in the bone marrow. A similar process occurs with avian heterophils.

2. Bone marrow neutrophils conceptually may be divided into two compartments, although there is no physical distinction between them.
a. Proliferation or mitotic compartment
(1) Myeloblasts, promyelocytes, and myelocytes are capable of mitosis and comprise this compartment.
(2) Four to five cell divisions occur, producing 16 to 32 cells from one differentiating stem cell. There are three mitoses in the myelocyte stage; therefore, most of the increase in cell numbers occurs at this stage.
(3) Maturation occurs concurrently with proliferation in this compartment.
(4) The transit time in this compartment is approximately two and a half days.
(5) In health, approximately 20% of the neutrophils in the bone marrow are in the proliferation compartment.
(6) Up to 20% of granulopoiesis is ineffective in the healthy dog. Premature death of cells, usually myelocytes, occurs. This degree of ineffective granulopoiesis has not been documented in other species.
b. Maturation and storage compartment
(1) Metamyelocytes, bands, and segmenters constitute this compartment. These cells cannot replicate but are functionally mature.
(2) The normal transit time in this compartment has been estimated to be two to three days in the dog, but can be shortened by early release of immature neutrophils into the blood during marked tissue demand for these cells.
(3) In health, approximately 80% of the neutrophils in bone marrow are in the storage compartment. This represents about a five-day supply of neutrophils in health, provided that the rate of cell turnover remains unchanged.
(4) Release of neutrophils from bone marrow into blood is orderly and age-related; the most mature cells (segmenters) are released first.
(5) During increased tissue demand for neutrophils, bands and younger forms may be released from the marrow prematurely, resulting in a left shift.

3. Mechanisms for increased neutrophil production
a. Increased stem cell recruitment
(1) This probably occurs at the earliest demand for neutrophils.
(2) Approximately three to five days are required for this increase to influence the number of blood neutrophils.
(3) Under appropriate conditions, bone marrow reserves of small animals are restored more quickly than those of large animals because of greater proliferative capacity.
b. Increased effective granulopoiesis
(1) Additional divisions may occur within the proliferation compartment; each additional division doubles the output of cells.

Free ebooks ==> www.Ebook777.com

(2) Increased output of cells may occur by reducing myelocyte attrition (ineffective granulopoiesis), which normally occurs in healthy dogs.
(3) This effect is realized in the blood in two to three days.

c. Shortened marrow transit or maturation time
(1) Neutrophils are released from the bone marrow into the blood at a faster rate.
(2) Increased mitosis of neutrophil progenitor cells and shortened maturation of their progeny in the bone marrow may temporarily meet increased tissue demand for neutrophils.

4. Neutrophil release from the bone marrow
a. Release is mediated by G-CSF, GM-CSF, C5a, tumor necrosis factor (TNF-α and TNF-δ), and, possibly, a cleavage factor of the third component of complement, which are derived from macrophages and other cells.
b. Increased rate of release from the storage compartment causes the rapidly developing neutrophilia (earlier than two days) that follows initial tissue demand for neutrophils.
c. The granulocytic marrow at this point exhibits a shift toward immaturity with a relative increase in the proliferation compartment because mature cells (segmenters) have been removed from the storage pool.
d. As tissue demand for neutrophils intensifies and the storage reserve of segmenters diminishes, band neutrophils appear in the blood (left shift). In severe situations, they may be followed by neutrophilic metamyelocytes (juveniles) or even more immature cells. A left shift indicates a decreased granulocyte reserve and usually denotes a tissue demand for neutrophils.

D. Neutrophil kinetics in blood
1. Neutrophils move more slowly than erythrocytes and plasma within postcapillary venules because of adhesion molecules on both neutrophils and endothelial cells. This causes an uneven distribution of neutrophils within the blood vasculature. The concentration of neutrophils in post capillary venules is greater than that in large-vessel blood.
a. Neutrophils that are hesitatingly adherent to the vascular endothelium constitute the marginal neutrophil pool (MNP) and are not quantitated in the routine white blood cell (WBC) count.
b. Neutrophils moving as fast as erythrocytes and plasma in the axial or central blood flow of arteries and veins make up the circulating neutrophil pool (CNP).
(1) Neutrophil numbers derived from the routine WBC count and differential count represent the approximate size of the CNP.
(2) CNP plus MNP equals the total blood neutrophil pool (TBNP).
c. The MNP is equal to the CNP in the dog, horse, and calf. The feline MNP is approximately three times larger than the CNP.
2. The average transit time for a neutrophil in blood in health is approximately 10 hours.
3. All blood neutrophils are replaced approximately two and a half times each day.
4. Neutrophils emigrate randomly from the blood into tissue spaces, unaffected by cell age. Migration is unidirectional; they do not return to circulation.
5. In health, neutrophils can survive in the tissues for 24 to 48 hours. Ultimately, effete neutrophils are largely removed by macrophages of the spleen, liver, bone marrow, and other tissues. Some neutrophils may be lost from the body via secretions, excretions, or transmigration of mucous membranes.

E. Function
1. Phagocytosis and microbicidal action are the primary functions of neutrophils. This activity is conducted efficiently in tissue but not in blood.
a. Metabolically active processes for these functions include the following:
(1) Adherence to and emigration through the vessel wall
(2) Chemotaxis, a motile response toward an attractant (e.g., C5a, bacterial products, arachidonic acid metabolites) in the tissue

www.Ebook777.com

(3) Ingestion and degranulation
(4) Bacterial killing

b. These functions may be compromised by deficiencies of various humoral or cellular components, drugs, or toxic bacterial products, increasing susceptibility to disease.

2. When exposed to bacteria or their products, neutrophils undergo a respiratory burst and secrete certain substances that may have the following functions:
   a. Extracellular digestion of fibrinogen and complement components
   b. Stimulation of the generation of inflammatory mediators
3. Neutrophils also may contribute to pathologic events of certain conditions (e.g., immune-complex glomerulonephritis and rheumatoid arthritis) by releasing inflammatory mediators into the surrounding tissues.
4. Abnormalities in neutrophil function involving adherence, chemotaxis, phagocytosis, and bacterial killing have been described in animals. These abnormalities in neutrophil function may predispose affected animals to disease.
5. Besides bactericidal activity, neutrophils can kill or inactivate some fungi, yeasts, algae, parasites, and viruses.
6. Other functions of neutrophils may include elimination of transformed cells, amplification and modulation of acute inflammation, and minor involvement in regulation of granulopoiesis.

II. MONOCYTES-MACROPHAGES

A. Morphology (Figure 2.1)
   1. Monocyte. The maturation sequence of the monocyte includes the following:
      a. Monoblast
         (1) This cell is of low frequency in the bone marrow.
         (2) It is indistinguishable from the myeloblast with Romanowsky stains.
      b. Promonocyte
         (1) The nucleus is indented and irregular with finely stranded chromatin.
         (2) Ultrastructurally, there are bundled or scattered cytoplasmic filaments.
      c. Monocyte
         (1) As viewed in Romanowsky-stained blood films, monocytes are generally the largest leukocytes circulating in health.
         (2) The nucleus is oval, reniform, bilobed, or trilobed with lacelike chromatin.
         (3) The cytoplasm is blue-gray (darker than that of immature neutrophils such as myelocytes, metamyelocytes, and bands), granular, and may or may not have vacuoles.
            (a) Distinct, variably sized, round vacuoles in monocytes commonly are an artifact of prolonged *in vitro* storage of EDTA (ethylenediaminetetraacetate) anticoagulated blood prior to preparation of hematologic smears.
            (b) Vacuolation of monocytes in fresh blood smears may indicate cellular activation.
            (c) Monocyte vacuoles are relatively large and distinct compared with the frothy appearance of neutrophils with toxic vacuolation.
         (4) Pink cytoplasmic granules are lysosomes that contain peroxidase, acid hydrolases, α-naphthyl acetate and butyrate esterases, aryl sulfatase, and lysozyme.
         (5) Pseudopodia (short hair-like processes of the plasma membrane) may project from the cell margins.
         (6) Ultrastructural features include short profiles of rough endoplasmic reticulum (RER), lysosomes, vacuoles, and microfilament bundles.
   2. Macrophage
      a. Macrophages are derived from blood monocytes.

Free ebooks ==> www.Ebook777.com

b. Monocytes transformed into macrophages are rarely encountered in blood, but may be observed in capillary blood smears in disorders such as ehrlichiosis, histoplasmosis, leishmaniasis, and immune-mediated hemolytic anemia.

c. Macrophages contain more granules and proteolytic enzymes than monocytes.

d. Monocytes or macrophages recruited from the blood to sites of inflammation are short-lived, whereas resident tissue macrophages may survive for prolonged periods (weeks to months), are capable of division, and are functional phagocytes.

e. Monocyte-derived macrophages include the following:

(1) Macrophages or histiocytes of exudates

(2) Pleural and peritoneal macrophages

(3) Pulmonary alveolar macrophages

(4) Connective tissue histiocytes

(5) Macrophages of the spleen, lymph nodes, and bone marrow

(6) Kupffer cells of the liver

f. Monocyte-macrophage cells constitute the so-called "reticuloendothelial system"; however, these cells do not produce reticular fibers and are not endothelial cells.

B. Production and kinetics

1. Monocytes are derived from a bipotential progenitor cell (CFU-GM) that is common to both monocytes and neutrophils.

2. Monocyte production and maturation are regulated by growth factors and cytokines, including SCF, M-CSF, GM-CSF, IL-1, IL-3, and IL-6.

3. There are probably at least three divisions from monoblast to the last promonocyte division.

4. Maturation is rapid, requiring only 24 to 36 hours.

5. Monocytes are released directly into blood from a promonocyte proliferation pool in bone marrow shortly after the last division (equivalent in age to the first-generation neutrophil myelocytes). There is essentially no marrow storage pool for monocytes as there is for neutrophils.

6. Evidence for a marginated monocyte pool exists.

7. The average blood transit time of monocytes is longer than for neutrophils, ranging from approximately 18 to 23 hours.

C. Function

1. Monocytes phagocytize and digest foreign particulate material and dead or effete cells.

2. Monocytes are less efficient phagocytes than neutrophils in the defense against microbial invasion.

a. Certain microorganisms phagocytized by macrophages can survive and replicate in them (e.g., *Mycobacteria* spp., *Rickettsia* spp., *Leishmania* spp., and *Toxoplasma* spp.)

b. Macrophages can be activated by products of microorganisms, certain cytokines, and some inert agents. Activation consists of enhanced metabolism, lysosomal enzyme activity, mobility, and microbicidal and cytocidal activities.

3. Monocytes are a major source of CSFs and cytokines (e.g., G-CSF, M-CSF, IL-1, IL-3, TNF) involved in hematopoiesis.

4. Macrophages secrete a variety of substances that modulate the inflammatory response (e.g., chemotactic factors, plasminogen activator, collagenase, elastase, complement components, plasmin inhibitors).

5. Macrophages function in immune recognition by phagocytosing and processing foreign substances for antigen presentation to T lymphocytes.

6. Macrophages eliminate virus-infected and tumor cells via antibody-dependent cellular cytotoxicity.

7. Macrophages are actively pinocytotic and catabolize serum proteins.

www.Ebook777.com

III. EOSINOPHILS

A. Morphology (Figure 2.1)

1. Mature eosinophils have two to three nuclear lobes separated by constrictions.

2. Distinct, eosinophilic granules characterize the cell and become evident at the myelocyte stage of development.

a. These are the secondary or specific granules.

b. Secondary granules vary in size and shape among species (large and round in the horse, variably-sized and round in the dog, uniformly small and round in ruminants and pigs, rod-shaped in the cat, and moderately sized and round in most birds).

c. These granules are lysosomes that contain major basic protein, acid hydrolases, and an eosinophil-specific peroxidase, which play a significant role in eosinophil function. Major basic protein is located in the core of the granule, while peroxidase is located in the surrounding matrix of the granule.

d. Ultrastructurally, an electron-dense core or crystalloid may be observed in secondary granules of eosinophils from cats, dogs, horses, and some birds. Periodicity of the central core also may be present. Secondary granules of bovine eosinophils are homogeneous (lack crystalloids).

B. Production and kinetics

1. Eosinophil production and maturation in bone marrow parallels that of the neutrophil; eosinophils cannot be identified before the myelocyte stage of development. There is a storage pool of eosinophils.

2. IL-5 is the major cytokine that controls eosinophil production. It is most influential in eosinophil proliferation, differentiation, maturation, and function. GM-CSF and IL-3 may play minor roles in eosinophil development.

3. Bone marrow production requires two to six days.

4. The blood transit time is short ($T_{1/2}$ = 30 minutes in the dog), and a marginal pool of eosinophils exists.

5. Eosinophils emigrate from the blood and preferentially reside in subepithelial sites in the skin, lung, gastrointestinal tract, and endometrium. Minimal recirculation of eosinophils may occur.

6. In most avian species except raptors, eosinophils are the rarest leukocyte in circulation.

C. Function

1. Eosinophils attach to and kill helminths in a process mediated by antibody, complement, and T lymphocyte perforins. Eosinophils release major basic protein and generate toxic oxygen radicals via peroxidase activity.

2. Eosinophils may suppress hypersensitivity reactions. They are attracted by and inhibit chemical mediators liberated by mast cells during allergic and anaphylactic reactions.

3. Eosinophils may promote inflammation, especially in asthma and allergic disease. They bind to IgE and are activated by antigen-IgE complexes, releasing their granule contents which contribute to tissue damage in allergic reactions. Eosinophil recruitment is mediated by interleukins (IL-5, IL-2, IL-16).

4. Phagocytic and bactericidal capabilities are similar to those of neutrophils, but these processes are not as effective. Eosinophils are not protective against bacterial infections.

5. Tumor-associated eosinophilia has been reported with mast cell tumor, T-cell lymphoma, fibrosarcoma, and carcinoma in animals. Infiltration of neoplasms by eosinophils may be a positive prognostic indicator.

IV. BASOPHILS

A. Morphology (Figure 2.1)

Free ebooks ==> www.Ebook777.com

1. Mammalian basophils contain two to three nuclear lobes separated by constrictions or have a ribbon-like nucleus. Avian and reptilian basophils have a round nucleus.
2. Granules become evident at the myelocyte stage of development.
   a. Granules are round and stain metachromatically (purple) in most species.
   b. Feline basophils contain lavender, rod-shaped granules.
   c. Granules fill the cytoplasm of the cell in most species but are less numerous in the dog.
   d. Ultrastructurally, granules are often homogeneous; they cannot be differentiated from mast cell granules in most species. In some species, the granules may contain myelin figures, electron-lucent areas, or crystalloids; granule matrix dissolution may accompany poor fixation.
   e. Basophil granules contain histamine, heparin, and sulfated mucopolysaccharides, but lack acid hydrolases.

B. Production and kinetics
1. Maturation of basophils parallels that of the neutrophil; basophils cannot be identified before the myelocyte stage of development.
2. Basophils are sparse in most mammals but may be encountered more frequently in birds.
3. Production and release of basophils requires approximately two and a half days; bone marrow storage of these cells is minimal.
4. IL-3 is the major cytokine that governs basophil growth and differentiation. GM-CSF and IL-5 play minor roles in basophil development.
5. Despite similar functions, basophils arise independently of tissue mast cells and do not share a common basophil-mast cell progenitor.

C. Function
1. In differential leukocyte counts, basophils are usually present in small numbers if observed at all. Manual differential leukocyte counts give valid results only if basophil numbers are substantially elevated (2% or more of the leukocyte population). Automated differential leukocyte counts (e.g., ADVIA-120) can produce very accurate basophil counts.
2. Basophils and mast cells share many properties and serve similar functions. Specific functions of basophils include the following:
   a. Participation in immediate and delayed hypersensitivity reactions through mediator release (e.g., histamine release in allergic reactions)
   b. Promotion of lipid metabolism by activation of lipoprotein lipase
   c. Prevention and promotion of hemostasis via heparin release and kallikrein activity, respectively
   d. Rejection of parasites (e.g., ticks)
   e. Possible tumor cell cytotoxicity
3. The number of basophils may increase in certain myeloproliferative disorders.

V. MAST CELLS. MAST CELLS SHARE SIMILAR FUNCTIONS WITH BASOPHILS BUT CONSTITUTE A DISTINCT CELL LINE.

A. Morphology and production
1. Mature mast cells have a round nucleus and a moderate volume of cytoplasm filled with dark purple granules that often obscure the nucleus.
2. Mast cells are tissue leukocytes and are not found in the blood of normal healthy mammals.
3. Precursors are rare and unidentifiable in bone marrow. They move from marrow to blood to tissues as non-granulated mononuclear cells.
4. Once they establish tissue residence, mast cells undergo additional differentiation and growth.

B. Function
1. Mast cells predominantly reside in sub-epithelial locations (dermis, submucosa of gastrointestinal tract), but can be found associated with virtually any tissue (lymph nodes, liver, spleen)

www.Ebook777.com

2. Mast cells promote inflammatory reactions, especially hypersensitivity.
3. Mast cells are also involved in fibrosis.

C. Mastocytemia
1. Mastocytemia (increased mast cells in the blood) can indicate a reactive or neoplastic condition.
   a. An inflammatory leukogram typically accompanies reactive mastocytemia.
   b. Unless large numbers of mast cells are present in the blood, differentiation between reactive and neoplastic mastocytemia cannot be based on cell numbers alone.
2. Reactive conditions causing mastocytemia include enteritis (e.g., parvoviral enteritis), pleuritis, peritonitis, and hypersensitivity conditions.
3. Neoplastic mastocytemia indicates either systemic mast cell disease (commonly originating as a visceral or cutaneous mast cell tumor) or mast cell leukemia (rare).

VI. LYMPHOCYTES

A. Morphology (Figure 2.1)
1. Lymphocytes are differentiated by the presence or absence of lineage-specific cell surface antigens and antibody production, which can be detected by monoclonal antibodies. Only plasma cells and some cytotoxic T lymphocytes (granular lymphocytes) can be identified by morphology alone.
2. Mature lymphocytes
   a. Mature lymphocytes are small cells with scant amounts of blue cytoplasm. The nucleus is round with aggregated chromatin; a slight nuclear indentation may be present. Nucleoli usually are not visible with routine stains.
   b. Lymphocytes in the dog and occasionally in the cat and horse may have dark red cytoplasmic granules (granular lymphocytes) at the site of nuclear indentation. These are mainly cytotoxic T lymphocytes.
   c. Lymphocytes are the predominant circulating leukocyte in adult ruminants and many species of birds. Both small lymphocytes and larger lymphocytes with more abundant cytoplasm, indented nuclei, and lighter-staining chromatin occur in blood. Cytoplasmic granules are common in bovine lymphocytes.
   d. Storage in EDTA-anticoagulated blood for 30 to 60 minutes may cause lobulation of nuclei, cytoplasmic vacuolation, or smudging of lymphocytes on stained smears. Marked cellular swelling may be associated with prolonged blood storage.
   e. Mature lymphocytes are not necessarily end cells; on appropriate stimulation they may transform into immunoblasts and plasmacytoid cells. Transformation usually occurs in lymphoid tissues but occasionally may be observed in blood (reactive lymphocytes).
   f. Ultrastructurally, lymphocytes contain many free ribosomes. Plasma cells have an abundance of RER. Russell bodies, derived from RER, are dilated vesicles containing flocculent immunoglobulin.
3. Reactive lymphocytes (immunocytes or transformed lymphocytes) are occasionally encountered in low numbers in blood during periods of antigenic stimulation.
   a. These are probably T lymphocytes, but could be B lymphocytes.
   b. Reactive lymphocytes (immunocytes) are large cells with intensely basophilic cytoplasm, a pale Golgi zone, and/or fine cytoplasmic vacuoles. The nucleus has aggregated chromatin; nucleoli and nucleolar rings are usually absent.
4. Plasma cells represent the ultimate development of B lymphocytes in response to antigenic stimulation. They occur most frequently in lymph nodes, bone marrow, and other tissues. They are rarely observed in blood. Nuclear staining characteristics are the same as that of other reactive lymphocytes, but the cell is identifiable by an eccentrically placed, round nucleus with condensed chromatin and a pale Golgi zone in the cytoplasm.

Free ebooks ==> www.Ebook777.com

5. Lymphoblasts are large cells with a round nucleus, dispersed chromatin, multiple nucleoli or nucleolar rings, and a thin rim of blue, granular cytoplasm. They are not necessarily precursors or poorly differentiated cells, but represent pre- or post-mitotic stages in the lymphocyte cell cycle. They are rarely observed in blood except in acute lymphoblastic leukemia and lymphoma.

B. Production

1. The primary lymphoid organs are the thymus and bone marrow or bursa of Fabricius.

a. T lymphocytes are embryologically derived from the thymus and function in cell-mediated immunity.

b. B lymphocytes are embryologically derived from bone marrow of mammals or bursa of Fabricius in birds and function in humoral (antibody) immunity.

2. Most of the lymphocytes in adult mammals originate in the secondary lymphoid tissues including tonsils, lymph nodes, spleen, bronchial-associated lymphoid tissue (BALT), and gut-associated lymphoid tissue (GALT). The secondary lymphoid tissues of birds include spleen, conjunctival- or head-associated lymphoid tissue (CALT, HALT), GALT, and lymphoid aggregates in various other body tissues. Birds do not have lymph nodes.

3. Lymphopoiesis (i.e., lymphocyte division and transformation) occurs in the lymphoid tissues and depends upon the degree and type of antigenic stimulation as well as an array of interleukin (IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11) and cytokine (interferon-γ) influences.

4. Certain antigens stimulate B lymphocytes to divide and/or transform into effector cells that produce immunoglobulin (i.e., IgG, IgA, IgM, or IgE); plasma cells are the ultimate derivative of B lymphocytes.

5. Certain antigens stimulate T lymphocytes to divide and/or transform into effector (Th1/Th2) cells that produce lymphokines and mediate cellular immunity.

C. Lymphocyte distribution and circulation

1. Lymphocytes are distributed in lymph nodes, spleen, thymus, tonsils, GALT, BALT, bone marrow, and blood of mammals. Birds lack lymph nodes but have CALT, HALT, and lymphoid aggregates in many tissues and organs. GALT is especially prominent in waterfowl.

2. Compared with other leukocytes, lymphocytes are long-lived and are capable of mitosis and/or transformation to more functionally active forms.

a. The life span of lymphocytes is defined as the interval between successive mitoses or the time from the last mitosis until cell death.

b. Most lymphocytes are short-lived (about two weeks); however, other lymphocytes, such as memory cells, may have intermitotic intervals of weeks, months, or years.

3. Among the leukocytes, lymphocytes are unique because they recirculate.

a. The primary route of recirculation is efferent ducts of lymph nodes → thoracic duct or right lymphatic duct → blood → postcapillary venules of the cortex of lymph nodes → lymphoid parenchyma → efferent lymph again. Activation of receptors in postcapillary venules and subsequent cell binding is responsible for movement of lymphocytes from circulation into the tissues.

b. The recirculation of splenic lymphocytes is more direct, from blood → spleen → blood again.

c. Recirculation promotes antigen sensitization of naive lymphocytes and detection of transformed cells.

d. Recirculation is non-random. Lymphocytes exhibit "homing" to their tissue of origin in health. Normal recirculation patterns may be altered in disease.

e. The recirculation time ranges from approximately one to several hours, depending upon the cell type (T versus B lymphocyte) and route through tissues and organs.

f. The majority of recirculating lymphocytes are long-lived, memory, T cells.

g. Most of the B lymphocytes in blood are transient members of the recirculating lymphocyte population. The majority of B cells remain in lymphoid tissues.

www.Ebook777.com

h. Lymphocytes from GALT and BALT enter afferent lymph. Afferent lymph from other tissues is relatively cell-free in health, except for emigration of antigen-bearing macrophages draining from tissues to lymph nodes.

D. Function
- **1.** Antibody production
  - **a.** Antigenically stimulated B lymphocytes form plasma cells, which secrete immunoglobulins.
  - **b.** Macrophages and T lymphocytes also regulate antibody production.
- **2.** Regulatory activity
  - **a.** Appropriately stimulated T lymphocytes and, to a lesser extent, B lymphocytes secrete biologically active messenger molecules called interleukins, lymphokines, or cytokines.
  - **b.** Functions of lymphocyte-derived interleukins:
    - **(1)** Mediation of humoral immunity
    - **(2)** Mediation of cellular immunity
    - **(3)** Activation of inflammatory cells
    - **(4)** Regulation of lymphocyte production, activation, and differentiation
    - **(5)** Stimulation and regulation of hematopoiesis
- **3.** Cytotoxicity is a manifestation of cell-mediated immunity.
  - **a.** Certain T lymphocyte subsets (CD8+) are cytotoxic for virus-infected and transformed cells.
  - **b.** Natural killer cells (null cells) also are cytotoxic but do not require previous sensitization to perform this function.

LABORATORY EVALUATION OF LEUKOCYTES

I. WHITE BLOOD CELL COUNT

A. Methods of determination
- **1.** Manual counts are performed with prepackaged diluent (Unopette® system), hemocytometer, and microscope. Inherent error is approximately 20%, even with excellent technical skills. This method is preferred for avian blood cell counts.
- **2.** Automated cell counters
  - **a.** Results are more reproducible than by manual counting. Inherent error is approximately 5%, but it may be greater if the cell counts are extremely low or high.
  - **b.** Several blood abnormalities may introduce counting errors.
    - **(1)** Clumping of leukocytes or fragile leukocytes gives falsely low counts.
    - **(2)** Abnormally large or clumped platelets may falsely elevate the automated WBC count.
    - **(3)** Excessive Heinz bodies, especially in feline blood specimens, may clump and cause a falsely high WBC count. Heinz bodies do not disintegrate when the erythrocytes are lysed during the counting procedure.
    - **(4)** Erythrocyte lysing agents for some instruments may be too weak to completely lyse animal red cells, giving a falsely increased WBC count.
  - **c.** Normal feline platelets have the largest mean platelet volume (MPV) of domesticated animals. Platelets are not lysed during the leukocyte counting procedure and may falsely elevate the WBC count. This problem occurs more frequently in diseases in which increased platelet turnover is present; younger platelets have an even greater MPV.
  - **d.** In addition to correct standardization and quality control practices, a rapid check on the automated WBC count may be accomplished by comparing the estimated WBC count of a stained blood film (see below) to the WBC count generated by the instrument.
- **3.** All nucleated cells present, including nucleated red blood cells (nRBCs), are enumerated by manual or some automated counting methods.

Free ebooks ==> www.Ebook777.com

a. Nucleated erythrocytes are identified by examination of a stained blood smear; the number of nRBCs/100 leukocytes is recorded concurrently during the leukocyte differential count.

b. The WBC count is then corrected by the following formula:

$$\text{Corrected WBC count} = (\text{initial WBC count} \times 100) \div (100 + \text{nRBC})$$

4. The WBC count can be estimated from a well prepared, Romanowsky-stained blood film in emergency situations.

a. The estimated WBC count = (average number of leukocytes/45 to 50 × field of view) × (1,500)

b. Estimation of the WBC count also can be learned by comparing numbers of leukocytes in stained blood smears to known quantitative leukocyte counts until proficiency at estimating leukocyte numbers is achieved.

c. Estimated leukocyte counts are inferior to properly performed, quantitative WBC counts obtained manually or by an automated cell counter.

B. Abnormalities in the total WBC (leukocyte) count

1. Leukocytosis is an increase in the total WBC count. It is caused by increased numbers of any leukocyte; however, neutrophilia (increase in neutrophils) usually is responsible.

2. Leukopenia is a decrease in the total WBC count. It is usually caused by neutropenia (decrease in neutrophils), except in ruminants and some avian species in which lymphocytes predominate in the blood in health.

II. BLOOD SMEAR EVALUATION (FIGURE 2.1)

A. A systematic approach to microscopic evaluation of the blood smear is described in Chapter 1.

B. Differential leukocyte count

1. The Romanowsky-stained smear is examined with high dry (45×) or oil magnification (50 to 100×), and leukocytes are identified until 100 to 200 cells are classified by type. The number of nRBCs/100 leukocytes encountered should be tallied separately.

2. The percentage of a given leukocyte type multiplied by the WBC count gives the absolute number of that leukocyte type/μL of blood. As a cross check, the cumulative total of all the percentages of the various leukocyte subtypes should equal 100%. The cumulative total of the absolute cell counts for the various leukocyte subtypes should equal the total WBC count.

3. Interpretation of the leukogram should be based upon the absolute cell counts/μL of blood and not upon the relative percentages.

a. The suffix "philia" or "osis" (e.g., neutrophilia, eosinophilia, basophilia, lymphocytosis, and monocytosis) indicates an increase in number of a particular leukocyte.

b. The suffix "penia" or "cytopenia" (e.g., neutropenia, eosinopenia, basopenia, lymphopenia, and monocytopenia) indicates a decrease in number of a particular leukocyte.

4. Immature neutrophils are counted as part of the differential leukocyte count.

a. Bands are the usual type of immature neutrophil observed in a left shift, but earlier forms (metamyleocytes, myelocytes, etc.) are occasionally observed in severe disease.

b. Progressively more immature neutrophils (metamyelocytes, myelocytes, or promyelocytes) may be encountered in blood in certain circumstances (e.g., severe inflammation, myeloid leukemia).

c. A degenerative left shift is present if the number of bands and less mature neutrophils exceeds the number of segmented neutrophils.

C. Abnormal leukocyte morphology (Figure 2.1)

1. Toxic change of neutrophils (Cases 6, 8, 14, 21)

a. Toxic change can be associated with any inflammatory disease severe enough to cause accelerated neutrophil production and shortened maturation time in the marrow. Four manifestations of toxic change may occur in mammalian neutrophils:

www.Ebook777.com

(1) Cytoplasmic basophilia. The cytoplasm of toxic neutrophils has a diffuse blue color from retained ribosomes. This is the last form of toxic change to resolve with recovery from disease.
(2) Cytoplasmic vacuolation. This change often occurs concurrently with cytoplasmic basophilia, but cytoplasmic vacuolation (foamy appearance) is a more severe manifestation of toxic change. Vacuolation occurs during bacteremia and generalized infection in most species, but is not always specific for infection. This change results from cytoplasmic granule dissolution.
(3) Döhle bodies. These structures are blue to gray, angular, cytoplasmic inclusions representing retained aggregates of rough endoplasmic reticulum. They are most commonly observed in feline blood smears.
(4) Toxic granulation. This form of toxic change is infrequently observed in blood smears of horses and cats, representing severe toxemia. Toxic granulation is characterized by prominent pink-purple granulation of the cytoplasm. Altered membrane permeability of cytoplasmic primary granules permits uptake of Romanowsky stain. Toxic granulation should not be confused with prominent staining of secondary granules, which is not a sign of toxemia.

b. Toxic changes in avian heterophils include cytoplasmic basophilia, variable degranulation, vacuolation, and the presence of red and purple granules in the same cell.
c. Toxic neutrophils and heterophils may have compromised cell function.

2. Neutrophil nuclear hypersegmentation
a. Five or more distinct nuclear lobes are observed.
b. Causes of nuclear hypersegmentation of neutrophils:
(1) Prolonged blood transit time with corticosteroid therapy, hyperadrenocorticism, or late stages of chronic inflammatory disease
(2) Idiopathic finding in horses and goats
(3) Cobalt deficiency in cattle
(4) Hereditary macrocytosis in Poodles
(5) Abnormal vitamin B_{12} uptake in Giant Schnauzers
(6) Myelodysplastic syndrome and some forms of leukemia

3. Neutrophil nuclear hyposegmentation
a. Nuclear shape is typical of bands (parallel nuclear margins), metamyelocytes (slight nuclear indentation), or myelocytes (round to oval nucleus). The chromatin pattern suggests possible causes for this change in nuclear morphology.
(1) A less aggregated chromatin pattern may be observed in the typical left shift of infection, in leukemoid responses, and with some forms of granulocytic leukemia. Rarely, neutrophils may have a ring-shaped nucleus, indicating intense granulopoiesis or nuclear maturation abnormality
(2) A coarse, aggregated chromatin pattern may be observed in the following:
(a) Pelger-Huët anomaly. This hereditary condition has been observed in dogs (especially Australian Shepherds, Coonhounds, and Foxhounds), cats, rabbits, and a horse. The anomaly is transmitted as an autosomal dominant trait that may have incomplete penetrance in some dog breeds (Australian Shepherd). Eosinophils and basophils also have nuclear hyposegmentation. Cellular function is not impaired.
(b) Pseudo-Pelger-Huët anomaly has a similar appearance to the congenital anomaly but is acquired and transient. This condition may be associated with chronic infection, especially in cattle, and administration of certain drugs.

4. Asynchronous nuclear maturation. Disorderly nuclear maturation may accompany resurgent granulopoiesis, granulocytic leukemia, and myelodysplastic syndrome. This change is recognized by observing nuclear lobes with dispersed chromatin (indicating immaturity) that are connected by very thin constrictions or filaments (indicating maturity).

5. Reactive lymphocytes (immunocytes). These cells are often observed following antigenic stimulation (e.g., infection, vaccination).

Free ebooks ==> www.Ebook777.com

6. Lymphoblasts (immature lymphocytes). When observed in stained blood smears, these cells usually indicate malignant lymphoma with a leukemic blood profile or acute lymphoblastic leukemia.
7. Degranulated eosinophils. These cells are activated during disease and appear "moth eaten" because of cytoplasmic degranulation and vacuolation. Greyhounds may have vacuolated eosinophils in health.
8. Degranulated basophils. Affected cells appear vacuolated and lack purple granules. This change is especially noticeable in avian blood smears that are stained with Diff-Quik®.
9. Organisms or diagnostic inclusions may occasionally be observed in leukocytes on Romanowsky-stained blood and/or bone marrow smears. Examples include the following (Figure 2.3):

- **a.** Non-*Anaplasmataceae* spp. bacteria
  - **(1)** Intracellular rods and cocci are rarely seen within peripheral blood neutrophils during bacteremia.
- **b.** *Anaplasmataceae* spp. bacteria
  - **(1)** These organisms infect leukocytes and platelets and may be observed on peripheral blood smears. Organisms are most commonly observed early in infection.
  - **(2)** Different organisms within this family preferentially infect different hosts and different cell types.
    - ***(a)*** *Ehrlichia canis* infects mononuclear cells (monocytes, lymphocytes) of dogs (canine monocytic ehrlichiosis).
    - ***(b)*** *Ehrlichia ewingii* infects neutrophils and, rarely, eosinophils of dogs (canine granulocytic ehrlichiosis).
    - ***(c)*** *Anaplasma phagocytophilum* infects neutrophils and, rarely, eosinophils of dogs, horses, cattle, sheep, and goats.
    - ***(d)*** *Anaplasma platys* infects platelets of dogs.
  - **(3)** These organisms are small (0.5 µm) coccobacilli. After infecting a leukocyte or platelet, the organism replicates, producing a cluster of organisms called a morula.
- **c.** *Hepatozoon* spp.
  - **(1)** Gametocytes of this protozoan parasite primarily infect monocytes and neutrophils of mammals and erythrocytes of birds, reptiles, and amphibians.
  - **(2)** Severity of disease and degree of parasitism varies in dogs, depending on the *Hepatozoon* sp.
    - ***(a)*** *Hepatozoon canis* (South America, Europe, Asia, Africa) typically causes no to mild disease and large numbers of gametocytes can be found in peripheral leukocytes.
    - ***(b)*** *Hepatozoon americanum* (southern portions of North America) causes severe illness with only rare peripheral gametocytes.
  - **(3)** Gametocytes are lightly basophilic, oval organisms that measure 4 µm × 9 µm in size and contain a round, eccentrically placed nucleus.
- **d.** Canine distemper virus
  - **(1)** Distemper inclusions can be observed within erythrocytes and/or leukocytes early in infection (often before clinical signs of disease are evident).
  - **(2)** Inclusions are round to oval and range from 1 to 6 µm in size. Staining characteristics are variable as follows:
    - ***(a)*** Inclusions appear magenta to red with Diff-Quik® and traditional Romanowsky stains.
    - ***(b)*** Inclusions may appear pale blue with other rapid modifications of traditional Romanowsky-type stains.
- **e.** Other bacterial (e.g., *Mycobacteria* spp.), protozoal (e.g., *Toxoplasma* sp.), and fungal organisms (e.g., *Histoplasma* spp.) are rarely observed within peripheral leukocytes during disseminated infections.
- **f.** Several rare hereditary disorders may present with abnormal granulation or vacuolation of leukocytes.

www.Ebook777.com

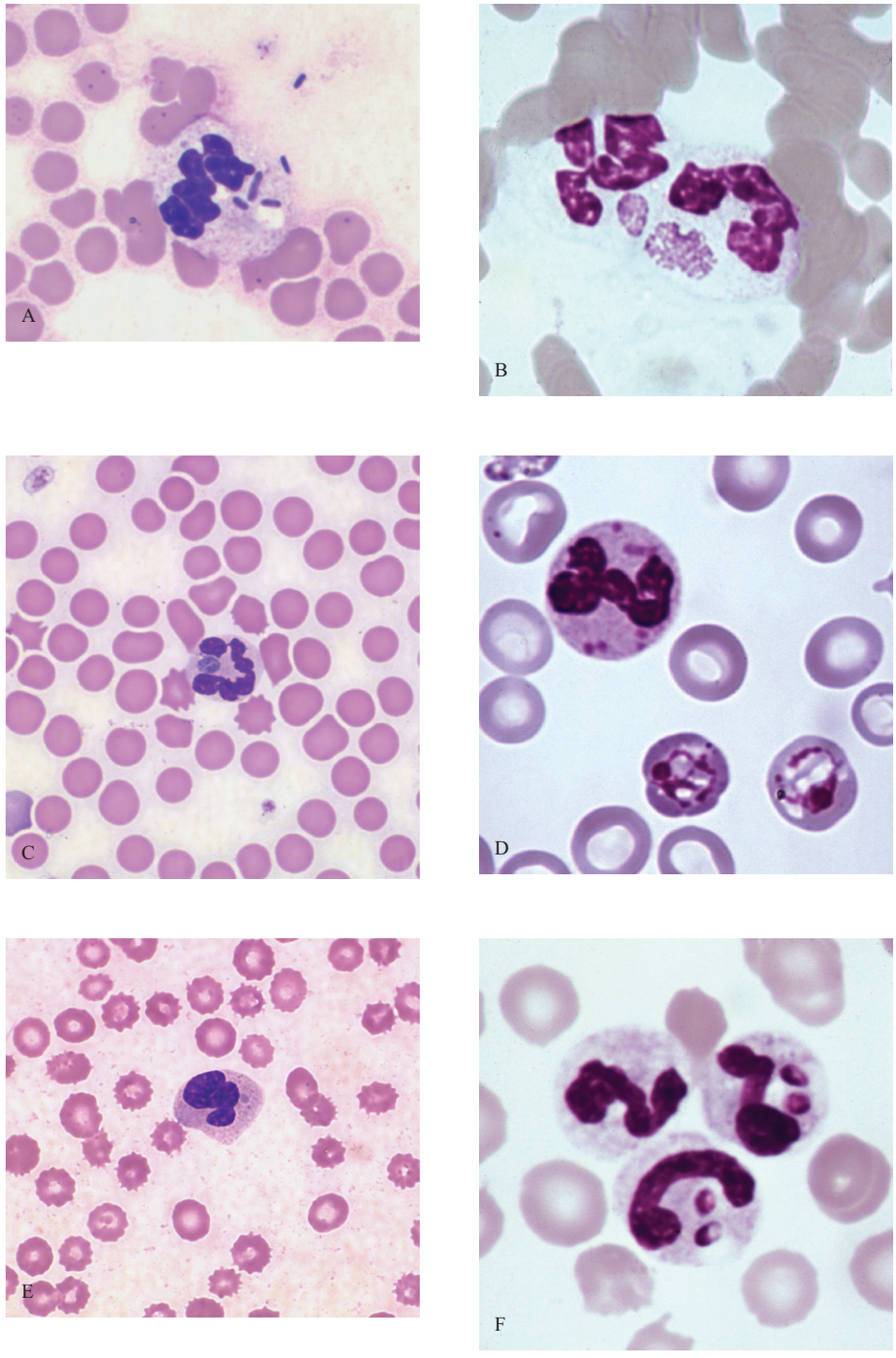


FIGURE 2.3. Infectious organisms within the cytoplasm of leukocytes. A. Equine neutrophil with intracellular and extracelluar bacilli (septic foal); B. *Anaplasma phagocytophilum* (previously *Ehrlichia equi*) morulae in cytoplasm of two neutrophils; C. *Ehrlichia ewingii* morula in the cytoplasm of a canine neutrophil; D. magenta canine distemper inclusions in a neutrophil and erythrocytes with traditional Wright-Leishman stain; E. pale blue cytoplasmic distemper inclusion in a canine neutrophil following staining with a rapid modification of Wright stain; F. *Histoplasma capsulatum* yeasts in cytoplasm of two of three canine neutrophils; continued.

Free ebooks ==> www.Ebook777.com

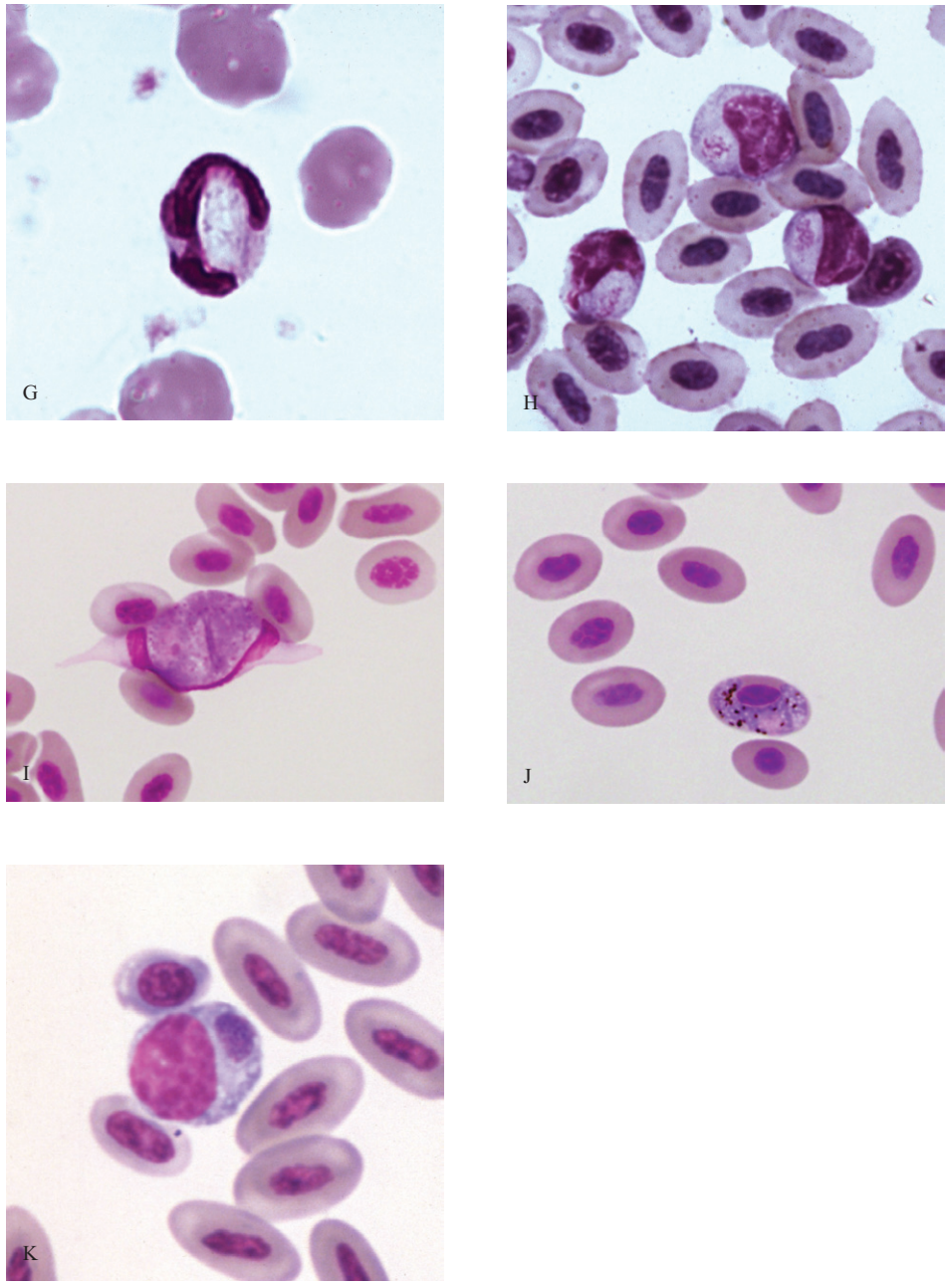


FIGURE 2.3. continued. G. *Hepatozoon canis* gametocyte in canine monocyte; H. *Atoxoplasma* sp. in cytoplasm of avian lymphocytes; I. *Leukocytozoon* sp. in an avian blood cell; J. *Hemoproteus* sp. in the cytoplasm of an avian erythrocyte; K. *Chlamydophila psittaci* (*Chlamydia psittaci*) inclusion in cytoplasm of avian monocyte.

www.Ebook777.com

(1) Lysosomal storage diseases may be characterized by an enzyme deficiency resulting in accumulation of undigestable substances within lysosomes. Examples include fucosidosis; GM1 and GM2 gangliosidoses; and mucopolysaccharidosis types IIIB, VI, and VII.
(2) Chediak-Higashi syndrome is characterized by abnormal fusion of lysosomes with eosinophilic granules (secondary granules) in the cytoplasm of neutrophils. This condition has been described in cattle, Persian cats, mink, beige mice, and rats.
(3) Birman cat neutrophil abnormality is characterized by prominent fine eosinophilic granulation within neutrophils. Granules are functionally normal.

III. BONE MARROW EXAMINATION. A SYSTEMATIC APPROACH TO EVALUATE BONE MARROW ASPIRATES IS PRESENTED IN CHAPTER 1.

INTERPRETATION OF LEUKOCYTE RESPONSES

The blood concentration of various granulocytes (neutrophils, eosinophils, basophils) and monocytes results from changes in the rate of release of these cells from the bone marrow into the blood, the distribution of these cells between circulating and marginal pools within the vasculature, and the rate of emigration of cells from the blood into tissues. In essence, granulocytes and monocytes within the blood are in transit from the bone marrow to the tissues where they will perform their functions. The concentration of lymphocytes in the blood, however, is primarily a reflection of changes in the kinetics of lymphocyte recirculation and cellular redistribution.

I. NEUTROPHILIA IN MAMMALS AND HETEROPHILIA IN BIRDS (TABLE 2.1, FIGURE 2.4)

A. Physiologic neutrophilia and heterophilia
  1. Leukogram findings
    a. Mild neutrophilia in mammals or heterophilia in birds is associated with up to a two-fold increase of absolute cell counts in the absence of a left shift.
    b. Lymphocytosis is present and may be more dramatic than the neutrophilia in some species (e.g. cat, birds)
    c. Monocyte, eosinophil, and basophil counts remain within the reference interval.
    d. The duration of physiologic leukocytosis is approximately 20 to 30 minutes.
  2. Possible clinical findings at the time of blood collection:
    a. A young animal that is healthy but has experienced excitement or fear.
    b. Evidence of increased heart rate, increased blood pressure, increased muscular activity, or seizures.
  3. Mechanism
    a. Epinephrine release in response to fear, excitement, or sudden exercise is responsible for physiologic neutrophilia and leukocytosis.
    b. The neutrophilia is a pseudoneutrophilia.
      (1) Increased heart rate, blood pressure, and blood flow cause mobilization of marginated neutrophils (MNP) with redistribution into the CNP.
      (2) Total blood neutrophil number (TBNP = CNP + MNP) is unchanged.
    c. The concomitant lymphocytosis is explained by two theories:
      (1) Epinephrine may block receptors on endothelial cells of postcapillary venules in lymph nodes, altering the normal recirculation pattern and preventing blood lymphocytes from re-entering lymphoid tissue.
      (2) An unidentified source of lymphocytes, possibly from the thoracic duct, is released into the general circulation in response to epinephrine.

Free ebooks ==> www.Ebook777.com

Table 2.1.
CAUSES OF NEUTROPHILIA IN MAMMALS AND HETEROPHILIA IN BIRDS.

Physiologic:
- Excitement
- Fear
- Exercise
- Convulsions
- Parturition

Corticosteroids:
- Exogenous (drug administration)
- Endogenous (stress, hyperadrenocorticism)

Inflammation (local or generalized):
- Infectious (primary or secondary):
  - Bacteria
  - Rickettsia
  - Viruses
  - Fungi
  - Parasites
- Non-infectious:
  - Burns
  - Infarction
  - Immune-mediated diseases
  - Necrosis
  - Postoperative
  - Thrombosis

Hemorrhage and hemolysis

Chemical and drug poisonings:
- Including early estrogen toxicosis in dogs and ferrets

Toxemia/toxicosis:
- Blue-green algal toxicosis
- Botulism
- Endotoxemia
- Uremia

Neoplasia:
- Granulocytic leukemia
- Myelomonocytic leukemia
- Other malignancies (including paraneoplastic syndromes)

Genetic disorders:
- Leukocyte adhesion deficiencies

4. Species characteristics
   a. Physiologic neutrophilia is uncommon in dogs.
   b. Physiologic leukocytosis is common in young healthy cats, where the absolute neutrophil count may exceed 39,000/µL and the absolute lymphocyte count may reach 36,000/µL. Neutrophilia is pronounced in the cat because of its larger MNP; however, the degree of lymphocytosis occasionally may exceed the magnitude of neutrophilia in some patients.
   c. The response may occur in healthy cattle during parturition and strenuous exercise. WBC counts are 15,000 to 27,000/µL. Neutrophilia and lymphocytosis occur, but unlike in other species, eosinopenia is expected.

www.Ebook777.com

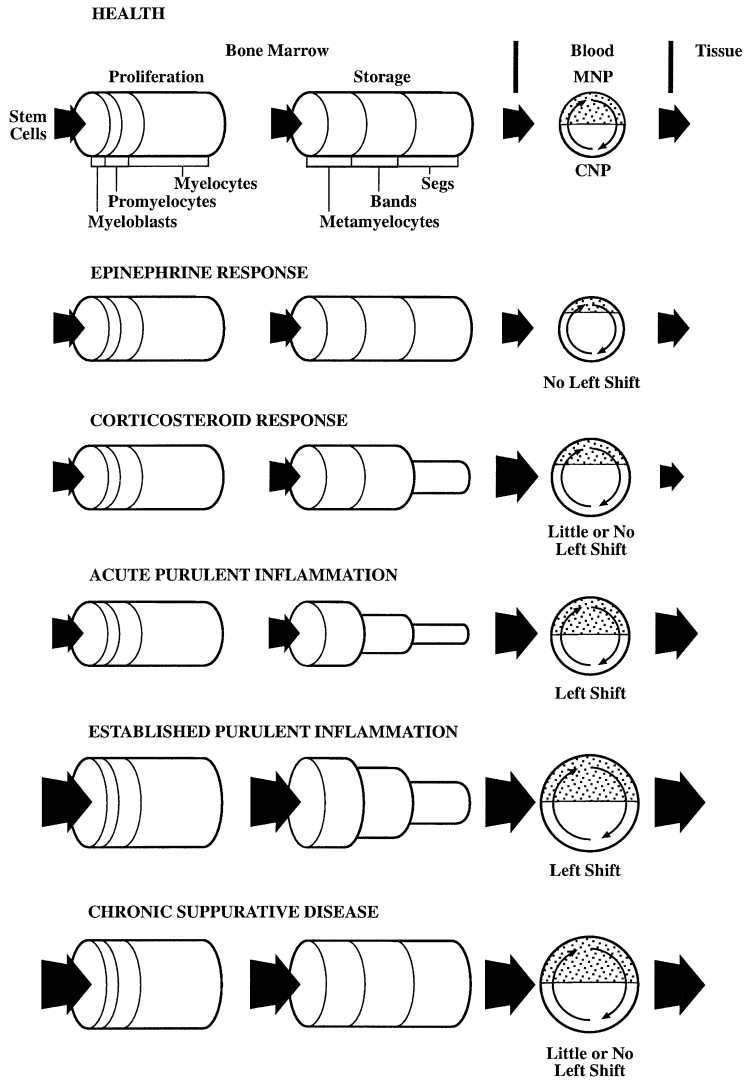


FIGURE 2.4. Mechanisms of neutrophilia. The sizes of the arrows represent the number of cells moving through the various compartments (stem cell, proliferation, storage, blood, and tissue). The sizes of tubes, circles, and shaded areas represent the relative sizes of the proliferation, storage, and various blood neutrophil pools, respectively.

d. Physiologic leukocytosis also is common in young healthy horses. The total WBC count may reach 26,000/µL. The absolute neutrophil count may exceed 14,000/µL, but the lymphocyte count seldom peaks above 14,400/µL.

e. Physiologic leukocytosis, especially lymphocytosis, accompanies lactation in sows, and it occurs in healthy pigs three to five hours after feeding.

f. Physiologic heterophilia is common in birds but relatively mild.

B. Corticosteroid-induced neutrophilia in mammals and heterophilia in birds (Cases 2, 3, 19, 20, 22, 23, 26, 27)

1. Leukogram findings

a. Neutrophilia in mammals or heterophilia in birds, usually without a left shift

Free ebooks ==> www.Ebook777.com

b. Lymphopenia
c. Eosinopenia
d. Monocytosis in the dog and occasionally in the cat

2. Possible clinical findings
a. Conditions that cause endogenous corticosteroid release (e.g., pain, high and low body temperatures, captive environment of birds)
b. Therapeutic use of corticosteroids
(1) The route of administration and dose affect the magnitude of the response.
(2) The peak response occurs four to eight hours after drug administration.
(3) The leukogram usually returns to the reference interval within 24 hours after a single injection of short-acting glucocorticoid and within two to three days after cessation of long-term therapy (10 or more days).
(4) The neutrophil count will return to the reference interval after several weeks of continuous corticosteroid therapy, but lymphopenia usually persists.
(5) In hyperadrenocorticism, the magnitude of the neutrophilia diminishes with longevity of the disease, but other changes in the leukogram persist.

3. Mechanisms
a. Neutrophilia is due to an increase in the TBNP caused by multiple mechanisms.
(1) Decreased emigration of neutrophils from circulation into the tissues and an increased blood transient time
(2) Increased bone marrow release of neutrophils. The number of cells released is usually not sufficient to deplete the storage pool of segmenters. Therefore, bands are not released and no left shift occurs.
(3) Decreased stickiness of neutrophils and a shift of cells from the MNP to the CNP
(4) Similar mechanisms are assumed to exist in birds.
b. Causes of lymphopenia
(1) Redistribution of recirculating lymphocytes; they remain transiently sequestered in the lymphoid tissues or bone marrow rather than entering efferent lymph and blood.
(2) Long-term usage of corticosteroids may cause lysis of thymic cortical lymphocytes and uncommitted lymphyocytes in the lymph nodes. Thymic medullary and bone marrow lymphocytes resist corticosteroid-induced lysis. Effector T- and B cells do not lyse.
c. Monocytosis may be caused by an effect similar to that on neutrophils (i.e., mobilization of marginated cells within the blood vasculature).
d. Causes of eosinopenia
(1) Margination or sequestration of eosinophils in the tissues. They resume circulation after cessation of the corticosteroid stimulation.
(2) Inhibition of release of eosinophils from bone marrow. Eosinophilopoiesis continues and the total number of marrow eosinophils increases during corticosteroid stimulation.
(3) Other possible mechanisms include inhibition of cytokines that govern eosinophil development and recruitment and induced apoptosis of eosinophils.

4. Species characteristics
a. Dog
(1) WBC counts are typically 15,000 to 25,000/µL, but may reach 40,000/µL on rare occasions.
(2) Neutrophilia without a left shift is typical, but immature neutrophils (up to 1,000/µL) may be released if the storage pool is depleted at the time of corticosteroid administration.
(3) Lymphocyte counts commonly are decreased (less than 1,000/µL).
(4) Monocytosis and eosinopenia are typically observed.
b. Cat
(1) The response is observed less frequently than in dogs.
(2) The leukogram pattern is similar to that of dogs, but monocytosis is infrequent.

www.Ebook777.com

(3) The most extreme leukocytosis is about 30,000/µL.

c. Cattle

(1) In addition to general causes of the corticosteroid release (pain and temperature extremes), the response may occur with abomasal displacement, mild fever, ketosis, dystocia, feed overload, and indigestion.

(2) WBC counts range from 8,000 to 18,000/µL.

(3) Eosinopenia caused by corticosteroids always occurs, but may be difficult to appreciate. Healthy cattle that are transported or placed in strange surroundings may have eosinopenia without other steroid-associated changes in the leukogram.

d. Horse

(1) This response may occur with sustained muscular exercise in healthy horses as well as with the usual causes of corticosteroid release.

(2) WBC counts range up to 20,000/µL.

(3) Lymphopenia is seldom dramatic; lymphocyte counts are usually within the lower end of the reference interval (2,000/µL in young horses and 1,500/µL in older horses).

e. Birds

(1) WBC counts may reach 35,000/µL.

(2) Moderate heterophilia may occur with counts ranging from 10,000 to 25,000/µL.

(3) Lymphopenia (2,500 to 9,000/µL) is most dramatic in avian species that have a predominance of lymphocytes in circulation in health.

C. Neutrophila and heterophilia of inflammation and infection (Cases 4, 7, 8, 11, 12, 17, 18).

1. Leukogram changes

a. Neutrophilia in mammals and heterophilia in birds. A left shift often is present.

(1) A left shift is the classic response to inflammation because the sudden demand for neutrophils or heterophils depletes the storage pool of segmenters, and bands are released.

(2) There are exceptions to the above generalization.

(a) Mild inflammation may not be severe enough to cause a left shift; thus, a mature neutrophilia may exist.

(b) In long-standing inflammation, production of neutrophils may be balanced with tissue usage; a left shift does not occur.

(c) Not all inflammation elicits a purulent response. Therefore, neutrophilia would not occur.

(d) During inflammation, endogenous release of cortisol in mammals or corticosterone in birds may secondarily cause neutrophilia or heterophilia, respectively, as described above.

b. Lymphopenia and eosinopenia

(1) These are common findings in inflammation or infection.

(2) Endogenous release of cortisol in mammals or corticosterone in birds may contribute to these changes.

(3) Because eosinophils are infrequent in the blood of cattle and birds (other than raptors), eosinopenia may be difficult to appreciate clinically in these species.

c. Monocytosis (Cases 8, 11, 14, 17)

(1) This is an inconsistent finding in inflammation and infection.

(2) Stress associated with inflammation may cause a steroid-induced monocytosis in the dog.

(3) Some forms of inflammation specifically elicit a monocytosis (e.g., granulomatous diseases, endocarditis).

2. Clinical findings

a. Obvious evidence of purulent or heterophilic inflammation may be present.

b. Purulent or heterophilic exudates may not be obvious with inflammation of the skin and mucosal surfaces because cells may be lost without much visible accumulation of matter.

c. Certain types of inflammation lack significant neutrophilic or heterophilic exudation (e.g., hemorrhagic cystitis, seborrheic dermatitis, catarrhal enteritis, certain granulomatous reactions). Neutrophilia and heterophilia may or may not develop.

Free ebooks ==> www.Ebook777.com

d. Localized purulent diseases such as pyometra or empyema stimulate greater neutrophilic responses than generalized infections or septicemias. Birds do not form pus; however, heterophil degranulation may provoke granuloma formation.

e. If a localized site of purulent or heterophilic inflammation is surgically extirpated, an exacerbation of neutrophilia or heterophilia should be anticipated during the immediate postsurgical period.

3. Mechanisms

a. Infectious agents and products of tissue injury stimulate a variety of cells to release interleukins, growth factors, cytokines, and other mediators of inflammation that are interrelated in causing neutrophil or heterophil proliferation, maturation, and release from the bone marrow into blood. These cells subsequently emigrate from the blood into tissues. The result of these activities on the numbers of blood neutrophils in mammals, or heterophils in birds, may be immediate or delayed for several days.

b. In mammals, an early, transient, endotoxin-mediated effect causes increased adhesiveness of neutrophils, retention of neutrophils in the MNP, and subsequent emigration into the tissues. This effect may result in a short-term neutropenia. Neutrophilia quickly follows because of an increased release of neutrophils from the bone marrow storage pool into the blood. Some birds appear to be refractory to the effects of endotoxin.

c. Following infection, inflammation, or endotoxin effects, cells in the neutrophil lineage are stimulated to proliferate and differentiate. These cells include the following:

(1) Multipotential stem cells

(2) Neutrophil progenitor cells (CFU-GM, CFU-G)

(3) Mitotic precursors in the bone marrow proliferative cell pool (myeloblasts, promyelocytes, and neutrophilic myelocytes)

d. The number of neutrophils in blood during purulent inflammation reflects a balance between the rate of release of cells from the bone marrow, the rate of emigration of cells from blood into tissues, and the rate of cell utilization within tissues (tissue demand).

(1) If bone marrow release is greater than cellular emigration into the tissues, neutrophilic leukocytosis occurs.

(2) If the emigration of cells from the vasculature exceeds the rate of replacement by the bone marrow (occurs with excessive tissue demand for neutrophils), neutropenia occurs.

(3) During purulent inflammation, a variable WBC count (from very low to very high) is possible, depending on this balance.

(4) A similar process explains the degree of heterophilia in avian patients.

e. With an increased tissue demand for neutrophils and an increase in the bone marrow release rate of these cells, the marrow storage pool may become depleted of segmenters. Band neutrophils and younger forms (neutrophilic metamyelocytes and myelocytes) may appear in blood following severe tissue demands for neutrophils.

(1) When the total neutrophil/heterophil count is within the reference interval or neutrophilia/heterophilia is present, a clinically important left shift exists when any of the following is present:

(a) Bands and other immature neutrophils are greater than 1,000/µL in dogs and cats.

(b) Bands and other immature neutrophils are greater than 300/µL in large animals.

(c) Bands and other immature heterophils are greater than 1,000/µL in birds. Left shifts are more difficult to identify in avian blood smears because heterophils have less nuclear lobulation and the prominent cytoplasmic granules may obscure nuclear morpohology.

(2) When neutropenia is present, greater than 10% bands and other immature neutrophils constitutes a clinically significant left shift in any species.

(3) A left shift is the hallmark of infection or severe inflammation; however, left shifts can occur in noninflammatory diseases (e.g., immune-mediated hemolytic anemia).

(4) The magnitude of the left shift tends to parallel the intensity of the purulent or heterophilic inflammation.

www.Ebook777.com

(5) In certain severe purulent diseases, marked neutrophilia may be accompanied by a left shift that includes myelocytes, progranulocytes, or myeloblasts in blood. This is called a "leukemoid response" because of its similarity to the blood profile of acute granulocytic leukemia.

f. During chronic, established, suppurative diseases, the rate of neutrophil production in the bone marrow eventually exceeds the rate of neutrophil release from the bone marrow and the rate of tissue utilization of neutrophils. As the marrow storage pool is replenished, the left shift diminishes or disappears despite a continued tissue demand for neutrophils. This mature neutrophilia persists until cell demand and production are balanced; the neutrophil count subsequently returns to the reference interval.

4. Species characteristics

a. Dog

(1) WBC counts between 10,000 and 30,000/µL are common. A few cases will have counts that exceed 50,000/µL, and in rare cases counts may exceed 100,000/µL.

(2) In contrast, neutropenia may result from overwhelming Gram-negative sepsis (e.g., salmonellosis, parvoviral enteritis) of the lung, thorax, peritoneum, intestine, or uterus. Endotoxemia may be a contributory factor.

(3) Leukemoid reactions and extreme neutrophilia may be associated with WBC counts exceeding 50,000 or even 100,000/µL. Examples include localized infection (e.g., pyometra, pyothorax, pancreatitis), certain forms of parasitism (e.g., hepatozoonosis), immune-mediated hemolytic anemia, and canine leukocyte adhesion deficiency. Extreme neutrophilia also has been associated with several neoplasms, typically carcinomas, as a paraneoplastic syndrome.

b. Cat

(1) WBC counts between 10,000 and 30,000/µL are common. A few cases have counts that exceed 30,000/µL, and in rare cases counts may exceed 75,000/µL.

(2) Gram-negative sepsis and endotoxemia cause neutropenia in cats as described for dogs.

c. Cattle

(1) Inflammatory leukograms in calves up to three or four months old may be similar to those described for cats and dogs, except that left shifts may be more subtle.

(2) Fibrinous, nonpurulent inflammation in cattle (e.g., early stages of shipping fever pneumonia) elicit little or no neutrophil response. Hyperfibrinogenemia (high plasma fibrinogen concentration) may be the first or only laboratory sign of inflammatory disease.

(3) In adult cattle, acute purulent inflammation frequently causes leukopenia, neutropenia, severe left shift, and lymphopenia (Case 21). This leukogram pattern lasts 24 to 48 hours. If the animal survives, neutrophil counts return toward the reference interval with a persisting left shift.

(4) Persistent purulent inflammation is associated with WBC counts ranging from 4,000 to 15,000/µL, with a predominance of neutrophils. Lymphocyte numbers are reduced by endogenous corticosteroid release during inflammatory disease.

(5) Infrequently, bovine patients have neutrophil counts that exceed 30,000/µl. In rare cases (e.g., empyema), counts may exceed 60,000/µL. Counts exceeding 100,000/µL may be observed in Holstein cattle with leukocyte adhesion deficiency. Because of the lack of proper adhesion molecule expression, neutrophils fail to emigrate from the vasculature and migrate to sites of tissue injury or infection.

d. Horse

(1) WBC counts are typically 7,000 to 20,000/µL. Rarely, the leukocyte count may exceed 30,000/µL.

(2) Left shifts associated with neutrophilia are generally moderate in the horse. Infrequently, diseases of the gastrointestinal tract associated with endotoxemia (e.g., acute salmonellosis) are characterized by a severe left shift with leukopenia or a total WBC count within the reference interval (Case 6).

Free ebooks ==> www.Ebook777.com

(3) Because neutrophil response to inflammation is frequently mild, hyperfibrinogenemia has been used as another indicator of inflammation.

e. Birds

(1) WBC counts are typically 24,000 to 45,000/μL. Occasionally, WBC counts may exceed 100,000 to 125,000/μL with salmonellosis, mycobacteriosis, or aspergillosis.

(2) Left shifts are more difficult to discern because avian heterophils have fewer nuclear lobes than mammalian neutrophils in health. Cytoplasmic granules also may obscure nuclear morphology.

(3) "Heteropil-lymphocyte reversal" may be observed in avian species with a predominance of circulating lymphocytes (as is the case in adult cattle).

D. Other causes of neutrophilia (Table 2.1)

1. Tissue necrosis and ischemia
2. Immune-mediated diseases with cell and tissue damage
3. Toxemia/toxicosis
4. Hemorrhage (Cases 1, 25)
5. Hemolysis (Cases 2, 3)
6. Neoplasia, including nonspecific malignancies and myeloproliferative disorders involving neutrophils

II. NEUTROPENIA IN MAMMALS AND HETEROPENIA IN BIRDS (TABLE 2.2, FIGURE 2.5)

A. Margination of circulating neutrophils (pseudoneutropenia)

1. Neutrophils shift from the CNP to MNP as blood flow slows or as adhesion molecules are expressed on the cell membranes of neutrophils and endothelial cells.
2. Endotoxin initially stimulates only margination of neutrophils (pseudoneutropenia); however, increased emigration of neutrophils from blood ultimately results in transition to a true neutropenia.
3. Birds may be refractory to these effects of endotoxin.

B. Excessive tissue demand for or destruction of neutrophils in mammals and heterophils in birds

1. Neutropenia occurs in inflammation or infection when the rate of emigration of neutrophils from the vasculature into the tissues exceeds the rate of replacement of these cells in the blood by the bone marrow (Cases 6, 21).
   a. Left shifts are common.
   b. Toxic change is likely, considering the causes of disease.
   c. Neutropenia occurs commonly in cattle, but does not carry as grave a prognosis as in other species.
   d. Neutropenia is found in peracute or acute inflammation before granulopoietic hyperplasia has time to occur in the bone marrow.
   e. Granulocytic hyperplasia in bone marrow usually follows with time and can have an effect on the WBC count within 48 to 72 hours.
   f. Similar changes occur with avian heterophils.
2. Immune-mediated destruction of circulating neutrophils with concurrent neutropenia is rare in animals. Neutropenia has been observed rarely with hemolytic disease of newborn foals and with drug administration. This mechanism has not been reported in birds.

C. Reduced production of neutrophils in mammals and heterophils in birds

1. Radiation, cytotoxic drugs used in cancer chemotherapy or immunosuppressive therapy, and some other drugs predictably cause neutropenia.

www.Ebook777.com

Table 2.2.
CAUSES OF NEUTROPENIA IN MAMMALS AND HETEROPENIA IN BIRDS.

Increased tissue demand:
- Bacterial infection
- Endotoxemia
- Immune-mediated diseases

Shifts from the circulating neutrophil pool to the marginal neutrophil pool:
- Endotoxemia (transient response and difficult to document)

Reduced production neutropenia:
- Cancer chemotherapeutic drugs and radiation
- Idiosyncratic drug reactions:
  - Antibiotics
  - Antimycotics
  - Estrogens
  - Nonsteroidal anti-inflammatory agents
  - rhGM-CSF
- Infectious agents:
  - Viruses
  - Rickettsia
  - Disseminated mycoses
- Toxicoses:
  - Bracken fern poisoning
  - Estrogen toxicity
  - Idiosyncratic drug reactions
- Myelophthisis:
  - Bone marrow necrosis
  - Myelofibrosis
  - Myelodysplastic syndrome
- Genetic disorders:
  - Canine cyclic hematopoiesis (Gray Collies)
  - Familial neutropenia (Standardbred horses, Belgian Tervuren dogs, Border Collies)
- Neoplasia:
  - Hematologic or metastatic neoplasia

a. Thrombocytopenia and anemia (pancytopenia or aplastic anemia) may follow.
b. Infections are considered imminent when neutrophil counts are 500/µL or lower.
c. Cytotoxic drugs also cause predictable heteropenia in birds.

2. Neutropenia is a common feature of diseases affecting hematopoietic stem cells (e.g., pancytopenia or aplastic anemia) (Case 4).

3. Certain viral and rickettsial infections
a. In mammals, a characteristic period of neutropenia may be present due to the death of progenitor cells and proliferating granulocytes in the bone marrow (Case 9) (e.g., feline infectious panleukopenia, canine parvoviral enteritis, feline leukemia virus [FeLV] and feline immunodeficiency virus [FIV] infections, ehrlichiosis). Endotoxemia secondary to enteric lesions also may play an important role in some viral infections.
b. In birds, heteropenia may be associated with circovirus, herpesvirus, polyomavirus, and reovirus infections.

Free ebooks ==> www.Ebook777.com

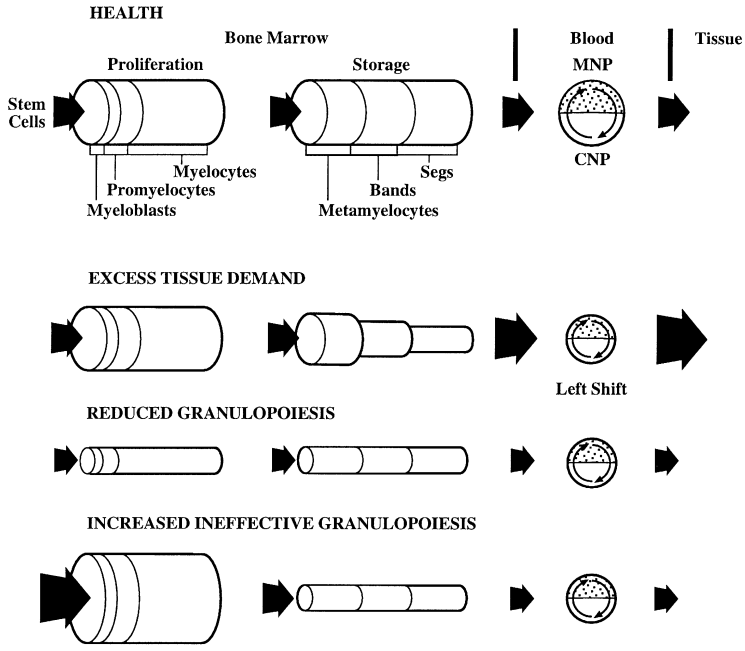


FIGURE 2.5. Mechanisms of neutropenia. The sizes of the arrows represent the number of cells moving through the various compartments (stem cell, proliferation, storage, blood, and tissue). The sizes of tubes, circles, and shaded areas represent the relative sizes of the proliferation, storage, and various blood neutrophil pools, respectively.

4. Diminished bone marrow production of granulocytes may be a transient feature during preclinical and acute stages of many viral diseases. Granulopoietic hyperplasia, reflecting recovery, may be encountered in bone marrow aspirates by the time neutropenia is observed.

5. Cyclic hematopoiesis (neutropenia) has been reported in animals.

a. Cyclic hematopoiesis of Gray Collie dogs has an autosomal recessive pattern of inheritance.

(1) Periods of neutropenia occur at 11- to 14-day intervals.

(2) The disease is presumed to result from a defect in stem cell regulation.

(3) Cyclic activity also affects erythrocytes and platelets.

b. Cyclic hematopoiesis occurs rarely in FeLV-infected cats.

c. Cyclophosphamide therapy may induce cyclic hematopoiesis in dogs.

6. Neutropenia may be a manifestation of idiosyncratic drug reaction (e.g., chloramphenicol in cats, phenylbutazone, cephalosporin antibiotics, griseofulvin, and estrogen toxicosis in dogs).

7. Inherited familial neutropenia has been observed in Standardbred horses and Belgian Tervuren dogs. The horses had signs of illness, suggesting that the neutropenia was pathologic. The dogs were clinically healthy, suggesting that their neutropenia was benign, as has been observed in some humans.

D. Excessive ineffective granulopoiesis

1. This condition is uncommon except in cats with FeLV infection and myelodysplastic syndrome (Chapter 3).

2. Bone marrow examination reveals granulocytic hyperplasia concomitant with neutropenia.

3. The bone marrow proliferating pool is expanded with a concomitant decrease in the maturation and storage pool of neutrophils, giving the appearance of a shift toward immaturity. A predominance of promyelocytes suggests a "maturation arrest" of neutrophil development.

www.Ebook777.com

III. MONOCYTOSIS

A. Monocytosis can occur any time that neutrophilia occurs, because both cell lines are derived from a common bipotential stem cell.

B. Monocytosis is the least characteristic change in the leukogram in response to corticosteroids, except in the dog (Cases 2, 26).

C. Monocytosis may be observed in both acute and chronic stages of disease (Cases 8, 11, 14, 17).

D. Monocytosis heralds the recovery from neutropenia. Because there is no bone marrow storage pool for monocytes, these cells are released into the blood at an earlier age than neutrophils.

E. Monocytosis may be the most prominent change in the leukogram during bacterial endocarditis and bacteremia.

F. Disorders characterized by suppuration, necrosis, malignancy, hemolysis (Case 2), hemorrhage (Case 25), immune-mediated injury, and certain pyogranulomatous diseases may be associated with monocytosis. Marked monocytosis also may be observed in birds with chlamydophilosis (chlamydiosis, ornithosis, psittacosis). This disease is caused by *Chlamydophila psittaci* (formerly known as *Chlamydia psittaci*), an obligate, intracellular, Gram-negative bacterium that lacks a cell wall.

IV. MONOCYTOPENIA

A. This finding is not a clinically useful feature of leukograms.

V. EOSINOPHILIA (TABLE 2.3)

A. Eosinophilia generally is associated with parasitic infection or hypersensitivity (Case 27).

B. Animals with eosinophilic disorders may have a response tempered by a concomitant corticosteroid effect (an eosinopenic effect).

C. Frequently, localized lesions that contain significant numbers of eosinophils in the exudate are not accompanied by eosinophilia in the blood (e.g., eosinophilic granuloma).

D. Antigens that stimulate eosinophilia mediate the response via sensitized T lymphocytes. The second exposure to antigen yields more rapidly developing and intense eosinophilia analogous to an immune (antibody) response.

1. The tissues most commonly affected in eosinophilic hypersensitivity conditions are mast cell rich and include skin, lung, gastrointestinal tract, and uterus.
2. Endo- and ectoparasites with prolonged host-tissue contact promote the most dramatic eosinophilia.

E. An allergic pathogenesis has not yet been demonstrated for all eosinophilic inflammatory conditions (e.g., eosinophilic granuloma).

F. Hypereosinophilic syndrome, especially in cats and Rottweiler dogs, may be impossible to distinguish from eosinophilic leukemia. Both diseases are characterized by persistent, often intense, eosinophilia with cellular infiltration of various tissues and organs.

G. Eosinophilia in birds is unusual and is most frequently observed in raptors. Dietary exposure to parasites may be involved.

Free ebooks ==> www.Ebook777.com

Table 2.3.
CAUSES OF EOSINOPHILIA.

- Parasitism:
  - Ectoparasites:
    - Arthropods
  - Endoparasites:
    - Nematodes
    - Protozoa
    - Trematodes
- Immediate or delayed hypersensitivity:
  - Asthma
  - Dermatitis
  - Eosinophilic granuloma
  - Feline eosinophilic keratitis
  - Gastroenteritis
  - Pneumonitis
  - Milk hypersensitivity in cattle
  - Canine panosteitis
- Neoplasia:
  - Primary:
    - Eosinophilic leukemia
  - Paraneoplastic:
    - Mast cell tumor
    - T-cell lymphoma
    - Lymphomatoid granulomatosis
    - Various carcinomas
    - Fibrosarcoma
    - Thymoma
- Infections:
  - Virus (some strains of FeLV)
  - Bacteria (some staphylococci and streptococci)
  - Fungi (cryptococcosis)
  - Slime molds (pythiosis)
- Drug reactions:
  - Tetracycline
  - IL-2
- Miscellaneous:
  - Hypereosinophilic syndrome (cats, Rottweiler dogs)
  - Hypoadrenocorticism (fewer than 20% of patients)
  - Hyperthyroidism (cats)

VI. EOSINOPENIA

A. Eosinopenia occurs in response to corticosteroids via cell redistribution in the vasculature. Other proposed mechanisms include inhibition of mast cell degranulation, neutralization of histamine in the circulation, or infiltration of lymphoid tissues subsequent to corticosteroid-associated lympholysis and cytokine release.

B. Catecholamine (epinephrine) release promotes eosinopenia by a δ-adrenergic effect.

www.Ebook777.com

C. The etiology of eosinopenia in acute infection occurs by a mechanism independent of corticosteroid action.

D. Eosinophils are rare in the blood of avian species other than raptors. Therefore, eosinopenia may be difficult to appreciate clinically.

VII. BASOPHILIA (TABLE 2.4)

A. An inverse relationship exists between the number of circulating basophils and tissue mast cells. Basophils are rare in the blood of mammals that have a rich tissue mast cell supply. Conversely, basophils are more prominent in avian blood while tissue mast cells are sparse.

B. Basophilia in mammalian blood smears is seldom dramatic, but observing even a few of these cells on the blood smear usually attracts attention. Basophilia is present when these cells exceed 200 to 300/μL.

Table 2.4.
CAUSES OF BASOPHILIA.

Parasitism:
- *Dirofilaria immitis* infestation (dogs and cats)
- *Acanthocheilonema reconditum* infestation
- Hepatozoonosis
- Ancylostomiasis (dogs)
- Schistosomes
- Ticks
- Air sac mites (birds)

Allergic diseases:
- Dermatitis
- Pneumonitis
- Eosinophilic granulomas
- Gastroenteritis
- Some bacterial infections (birds)

Drug reactions:
- Heparin
- Penicillin

Stress:
- Feed restriction (birds)
- Starvation (birds)
- Forced molting (birds)
- Ingestion of mycotoxins (birds)

Neoplastic diseases:
- Mast cell tumor
- Myeloproliferative disease
- Thymoma
- Lymphomatoid granulomatosis
- Essential thrombocythemia

Basophilic leukemia

Free ebooks ==> www.Ebook777.com

C. Those IgE-generating disorders that cause predictable eosinophilia often have concomitant basophilia.

D. Basophilia occurs in cats, but it may be overlooked because the granules of mature basophils do not stain metachromatically. The granules usually appear lavender to taupe (brownish-gray) with Romanowsky stains.

E. Basophilia in the absence of eosinophilia is rare, but may be observed occasionally in equine blood smears.

F. Disorders of lipid metabolism in mammals are not associated with basophilia, as measured by the routine WBC count. The routine WBC count is too crude to quantitate very mild basophililia precisely.

G. Basophilia is observed more commonly in avian blood smears, probably because of increased numbers of these cells in health. However, avian basophils may be difficult to identify because their granules may dissolve with Diff-Quik® staining. Degranulated basophils have a vacuolated or "moth-eaten" appearance.

VIII. BASOPENIA

A. Basopenia is difficult to appreciate clinically unless special stains, diluent, and a hemacytometer are used to perform an absolute basophil count.

IX. LYMPHOCYTOSIS (TABLE 2.5)

A. The number of circulating lymphocytes tends to be quite constant in health and decreases slightly with age in most species; young animals have higher lymphocyte counts. For example, average lymphocyte counts in horses decline from approximately 5,200 to 3,100/μL between 8 months and over 5 years of age.

B. The epinephrine effect of excited, healthy animals causes lymphocytosis as described earlier in the chapter (Section I, A).

Table 2.5.
CAUSES OF LYMPHOCYTOSIS.

Physiologic response:
Young animals, especially cats and birds
Chronic antigenic stimulation:
Bacterial infection
Rickettsial infection
Viral infection
Deep mycosis
Protozoal infections
Post-vaccination
Hypoadrenocorticism
Lymphoid neoplasia:
Lymphoma
Lymphoid leukemia
Thymoma

www.Ebook777.com

C. Antigenic stimulation occasionally causes lymphocytosis.

1. Enlarged lymph nodes caused by antigenic stimulation (lymphoid hyperplasia) are commonly encountered in all species, but blood lymphocyte numbers correlate poorly with increased functional reactivity.

2. The blood lymphocyte number may be within the reference interval or decreased in association with enlarged lymph nodes, especially during acute stages of infection (see following discussion on lymphopenia).

3. Reactive lymphocytes (immunocytes) may occur in the blood with or without lymphocytosis.

4. During chronic stages of infection, intensifying lymphocytosis (up to 20,000 to 30,000/μL) may be seen infrequently (e.g., Rocky Mountain Spotted Fever and canine ehrlichiosis) in dogs. Rickettsial titers should be evaluated to distinguish reactive lymphocytosis from chronic lymphocytic leukemia.

D. Persistent lymphocytosis in cattle is a subclinical, nonneoplastic manifestation of bovine leukemia virus infection (Chapter 3). Viral infection promotes B lymphocyte hyperplasia; lymphocyte counts typically range from 7,000 to 15,000/μL.

E. Lymphocytosis is common in lymphocytic leukemias and can occur in association with the leukemic blood profile of lymphoma.

F. Lymphocyte counts in healthy growing swine are higher than other species, typically 13,000 to 26,000/μL. Lymphocyte counts rarely exceed reference values in swine.

G. Lymphocytosis may be dramatic in birds with chronic bacterial, viral, fungal, or parasitic infections.

X. LYMPHOPENIA (TABLE 2.6)

A. Lymphopenia is a common abnormality in the leukogram of sick animals. The mechanism leading to lymphopenia is seldom differentiated before a final diagnosis is made; it may be postulated only in retrospect.

B. Mechanisms that cause lymphopenia include:

1. Corticosteroid-induced redistribution of recirculating lymphocytes (Cases 2, 3, 20, 22, 26, 27)

2. Acute systemic infection. When there is generalized disbursement of infectious antigen, recirculating lymphocytes may be trapped in lymph nodes, which may be enlarged concomitant with lymphopenia. The lymphopenia tends to disappear with time.

3. Viral infections are more likely to cause lymphopenia than bacterial infections (Case 9).

4. Local infections may cause entrapment of lymphocytes in the regional lymph nodes, but lymphopenia is unlikely.

5. Acquired T-lymphocyte deficiency. Most recirculating lymphocytes are T cells. Certain infections in neonates cause thymic necrosis or atrophy; if the animal survives, persistent lymphopenia occurs.

6. Immunosuppressive therapy or irradiation. These agents suppress clonal proliferation of lymphocytes. Lymphopenia develops slowly.

7. Loss of lymphocyte-rich, efferent lymph. This mechanism of lymphopenia primarily occurs with loss of thoracic duct lymph (i.e., chylothorax).

8. Loss of lymphocyte-rich, afferent lymph. The only afferent lymph that is rich in cells comes from GALT or BALT. Lymphopenia may occur with intestinal lymphangiectasia (Case 16).

9. Disruption of lymph node architecture by inflammation, infection, or neoplasia that replaces lymphoid tissue and alters existing patterns of lymphocyte recirculation.

10. Hereditary immunodeficiency. Selective T-lymphocyte deficiency is characterized by lymphopenia, while combined T- and B-lymphocyte (SCID) deficiency have lymphopenia and decreased serum immunoglobulin concentrations. Animals with only B-lymphocyte deficiency do not have lymphopenia.

Free ebooks ==> www.Ebook777.com

Table 2.6.
CAUSES OF LYMPHOPENIA.

Drug-induced:
- Corticosteroids:
  - Exogenous corticosteroid or ACTH administration
  - Hyperadrenocorticism:
    - Endogenous cortisol production (mammals)
    - Endogenous corticosterone production (birds)
- Interleukin (rhIL-2)
- Colony-stimulating factor (rcG-CSF)

Acute systemic infection:
- Septicemia
- Endotoxemia
- Viruses (often only in very early stages)

Therapeutically induced:
- Immunosuppressive drugs
- Chemotherapeutic drugs
- Radiation

Loss of lymphocyte-rich lymph:
- Efferent lymph:
  - Chylothorax
  - Feline cardiac disease
- Afferent lymph:
  - Alimentary lymphoma
  - Enteric neoplasms
  - Granulomatous enteritis, including paratuberculosis
  - Protein-losing enteropathy
  - Lymphangiectasia
  - Ulcerative enteritis

Disruption of lymphoid tissue architecture with altered lymphocyte recirculation:
- Generalized granulomatous diseases
- Multicentric lymphoma

Hereditary disorders:
- Selective T-lymphocyte deficiency
- Severe combined immunodeficiency (Arabian foals, Basset Hounds, Jack Russell Terriers)
- Thymic aplasia (Black-Pied Danish cattle)

XI. PROGNOSIS AND LEUKOCYTE RESPONSES

A. General considerations

1. A single leukogram does not reveal whether the clinical situation is improving or deteriorating.

a. Neutrophilia with a moderate left shift, where segmenters outnumber bands, may be an appropriate response at that point in time.

(1) The leukogram indicates that the infection or inflammation is severe and intense.

(2) However, the clinician may be unable to determine whether the neutrophil response is adequate to contain or eliminate the disease.

b. Neutrophilia wherein immature neutrophils outnumber segmenters (degenerative left shift)

(1) The leukogram indicates that overwhelming infection or inflammation is present.

www.Ebook777.com

(2) Intensification of the degenerative left shift suggests a poor prognosis, whereas resolution of the degenerative left shift suggests a more favorable prognosis.

2. Sequential leukograms usually are required for prognostic judgments.
3. Leukograms should be interpreted with a knowledge of historical data and clinical signs.
4. Leukogram interpretations should be based on the absolute cell counts. The relative leukocyte percentages, derived from the differential leukocyte count, may be misleading, particularly when the leukocyte in question represents only a small percentage of the total number of cells counted.

B. Favorable prognosis
1. If sequential leukograms exhibit a return toward reference intervals, a favorable prognosis is indicated when accompanied by convalescence in the animal.
2. Disappearance of a left shift indicates impending recovery and precedes the resolution of neutrophilia.
3. Disappearance of toxic changes in neutrophils suggests resolution of the inflammatory stimulus.
4. Resolution of lymphopenia or eosinopenia may precede clinical signs of recovery in the patient.

C. Guarded and poor prognosis
1. Neutrophil changes
a. Neutropenia, regardless of cause, is serious because of the increased risk of secondary bacterial infection.
b. A degenerative left shift, regardless of the total WBC count, implies an intense tissue demand for neutrophils that exceeds the ability of the bone marrow to replace these cells.
c. Extreme neutrophilic leukocytosis with or without a left shift to myelocytes or earlier neutrophil precursors (leukemoid response) indicates a guarded prognosis until granulocytic leukemia can be excluded and the disease process can be identified.
2. Lymphocyte changes
a. A declining lymphocyte count in an apparently healthy patient may indicate impending illness; however, impending illness probably cannot be distinguished from a corticosteroid effect.
b. Persistent lymphopenia suggests ongoing disease.
c. Marked lymphocytosis implies a guarded prognosis until the possibility of lymphocytic leukemia or lymphoma can be excluded.

REFERENCES

Andreasen CB, Roth JA: 2000. Neutrophil functional abnormalities. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 356–365.

Aroch I, Klement E, Segev G: 2005. Clinical, biochemical, and hematological characteristics, disease prevalence, and prognosis of dogs presenting with neutrophil cytoplasmic toxicity. *J Vet Intern Med* 19:64–73.

Baneth G, Macintire DK, Vincent-Johnson NA, et al: 2006. Hepatozoonosis. *In*: Greene CE (ed): *Infectious Diseases of the Dog and Cat*, 3rd ed. WB Saunders Co, St. Louis, pp. 698–710.

Bell TG, Butler KL, Sill HB, et al: 2002. Autosomal recessive severe combined immunodeficiency of Jack Russell terriers. *J Vet Diagn Invest* 14:194–204.

Bienzle D: Monocytes and macrophages. 2000. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 318–325.

Brown MR, Rogers KS: 2001. Neutropenia in dogs and cats: A retrospective study of 261 cases. *J Am Anim Hosp Assoc* 37:131–139.

Free ebooks ==> www.Ebook777.com

Cowell RL, Decker LS: 2000. Interpretation of feline leukocyte responses. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 382–390.

Duncan JR, Prasse KW, Mahaffey EA: 1999. *Veterinary Laboratory Medicine: Clinical Pathology*, 3rd ed. Iowa State University Press, Ames, pp. 37–62.

Evans EW: 2000. Interpretation of porcine leukocyte responses. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 411–416.

Gaunt SD: 2000. Extreme neutrophilic leukocytosis. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 347–349.

George JW, Snipes J, Lane VM: 2010. Comparison of bovine hematology reference intervals from 1957 to 2006. *Vet Clin Pathol* 39:138–148.

Gilbert RO, Rebhun WC, Kim CA, et al: 1993. Clinical manifestations of leukocyte adhesion deficiency in cattle: 14 cases (1977–1991). *J Am Vet Med Assoc* 202:445–449.

Greenfield CL, Messick JB, Solter PF, et al: 2000. Results of hematologic analyses and prevalence of physiologic leukopenia in Belgian Tervuren. *J Am Vet Med Assoc* 216:866–871.

Latimer KS, Bienzle D: 2000. Determination and interpretation of the avian leukogram. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 417–432.

Latimer KS, Campagnoli RP, Danilenko DM: 2000. Pelger-Huët anomaly in Australian shepherds: 87 cases (1991–1997). *Comp Haematol Int* 10:9–13.

Latimer KS: 1995. Leukocytes in health and disease. *In:* Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine. Disease of the Dog and Cat*, 4th ed. WB Saunders Co, Philadelphia, pp. 1892–1929.

Liska WD, MacEwen EG, Zaki FA, et al: 1979. Feline systemic mastocytosis: A review and results of splenectomy in seven cases. *J Am Anim Hosp Assoc* 15:589–597.

Madewell BR, Wilson DW, Hornof WJ, et al: 1990. Leukemoid blood response and bone infarcts in a dog with renal tubular adenocarcinoma. *J Am Vet Med Assoc* 197:1623–1625.

McManus PM: 1999. Frequency and severity of mastocytemia in dogs with and without mast cell tumors: 120 cases (1995–1997). *J Am Vet Med Assoc* 215:355–357.

Moore FN, Bender HS: 2000. Neutropenia. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 350–355.

Moore JN, Morris DD: 1992. Endotoxemia and septicemia in horses: Experimental and clinical correlates. *J Am Vet Med Assoc* 200:1903–1914.

Neer MT, Harrus S, Greig B, et al: 2006. Ehrlichiosis, Neorickettsiosis, Anaplasmosis and Wolbachia Infection. *In*: Greene CE (ed): *Infectious Diseases of the Dog and Cat*, 3rd ed. WB Saunders Co, St. Louis, pp. 698–710.

Raskin RE, Valenciano A: 2000. Cytochemistry of normal leukocytes. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 337–346.

Schultze AE: 2000. Interpretation of canine leukocyte responses. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 366–381.

www.Ebook777.com

Scott MA, Stockham SL: 2000. Basophils and mast cells. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 308–317.

Sellon RK, Rottman JB, Jordan HL, et al: 1992. Hypereosinophilia associated with transitional cell carcinoma in a cat. *J Am Vet Med Assoc* 201:591–593.

Shelton GH, Linenberger ML: 1995. Hematologic abnormalities associated with retroviral infections in the cat. *Semin Vet Med Surg (Small Animal)* 10:220–233.

Skubitz KM: 1999. Neutrophilic leukocytes. *In:* Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM (eds): *Wintrobe's Clinical Hematology*, 10th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 300–350.

Smith GS: 2000. Neutrophils. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 281–296.

Steffens WL: 2000. Ultrastructural features of leukocytes. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 326–336.

Sykes JE, Weiss DJ, Buoen LC, et al: 2001. Idiopathic hypereosinophilic syndrome in 3 Rottweilers. *J Vet Intern Med* 15:162–166.

Taylor JA: 2000. Leukocyte responses in ruminants. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 391–404.

Thompson JP, Christopher MM, Ellison GW, et al: 1992. Paraneoplastic leukocytosis associated with a rectal adenomatous polyp in a dog. *J Am Vet Med Assoc* 201:737–738.

Welles EG: 2000. Clinical interpretation of equine leukograms. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 405–410.

Wellman ML, Couto CG, Starkey RJ, et al: 1989. Lymphocytosis of large granular lymphocytes in three dogs. *Vet Pathol* 26:158–163.

Young KM: 2000. Eosinophils. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 297–307.

Free ebooks ==> www.Ebook777.com

CHAPTER 3

HEMATOPOIETIC NEOPLASIA

Dorothee Bienzle, DVM, MSc, PhD

Neoplasms arising from hematopoietic cells, especially lymphocytes, are among the most common manifestations of cancer in domestic animals (Figure 3.1). Various classification schemes are used to improve disease diagnosis, provide a meaningful prognosis, and choose an effective treatment protocol. Hematopoietic neoplasia can be broadly divided into two categories: neoplasia arising from lymphocytes (most commonly outside the bone marrow) and neoplasia arising from non-lymphoid (myeloid) cells in the bone marrow.

LYMPHOPROLIFERATIVE DISORDERS

In mammals, lymphoid neoplasms originate from clonal transformation of lymphocytes in lymph nodes, mucosal sites, bone marrow, spleen, thymus, and tissues lacking obvious lymphoid cell populations in health. In birds, lymphoid neoplasia may originate in any of the sites mentioned for mammals, except lymph nodes (birds do not have lymph nodes). In birds, lymphoid neoplasia also may originate in the bursa of Fabricius, a lymphoid-rich tissue adjacent to the cloaca.

Neoplasms may arise from lymphocytes with various degrees of differentiation and biological function, ranging from immature lymphocytes expressing few "cluster of differentiation" (CD) markers to terminally differentiated lymphocytes (Figure 3.1). The terminally differentiated lymphocytes include plasma cells, the ultimate stage of B lymphocyte development, and differentiated T lymphocytes with discrete CD marker expression. The various forms of lymphoid neoplasia include:

Lymphoma
Large granular lymphocyte (LGL) lymphoma
Plasma cell tumor
Acute lymphocytic leukemia
Chronic lymphocytic leukemia

I. LYMPHOMA

A. Definitions and general comments

1. Lymphoma is a neoplasm of lymphocytes arising as a solid tissue mass in organs other than bone marrow.

a. Lymphoma originally was called "lymphosarcoma." Lymphosarcoma probably is the most accurate name for this tumor of neoplastic lymphocytes.

b. In human medicine, the term lymphosarcoma subsequently was replaced by the term "malignant lymphoma." The suffix "oma" is used to designate a benign neoplasm. Therefore,

www.Ebook777.com

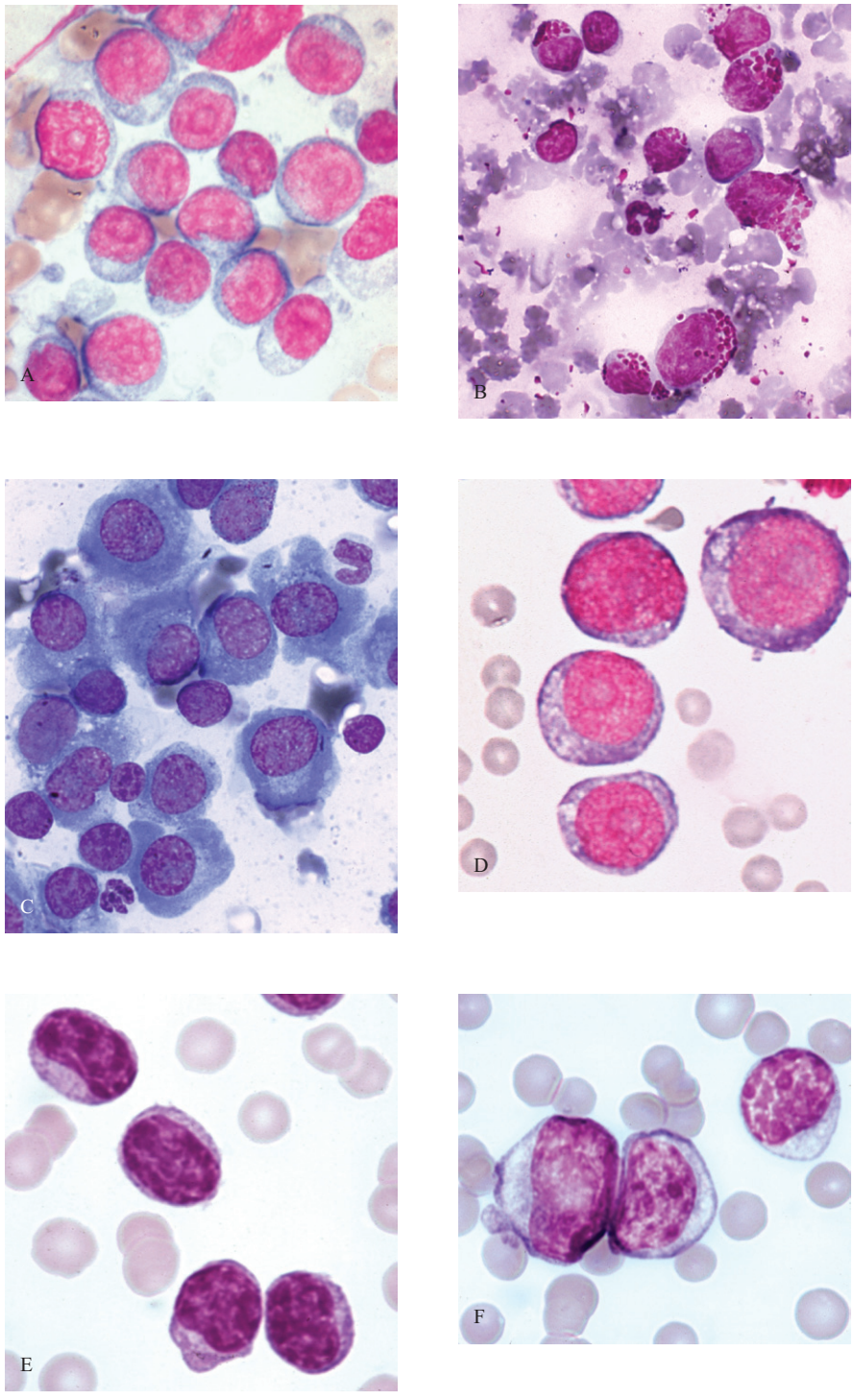
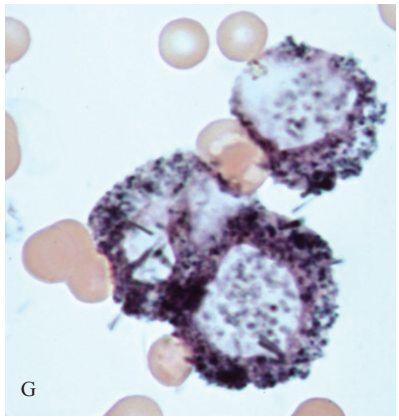
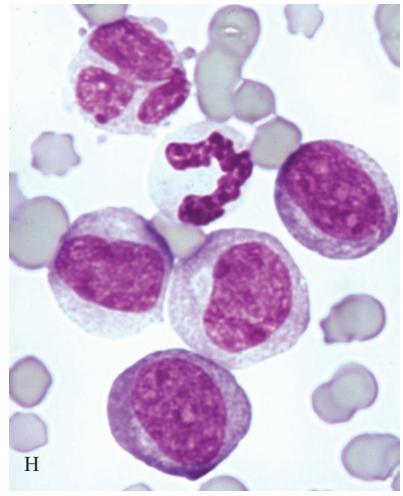
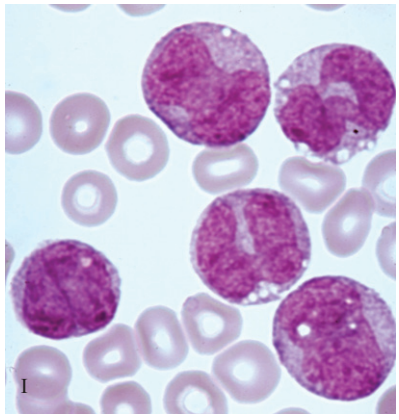


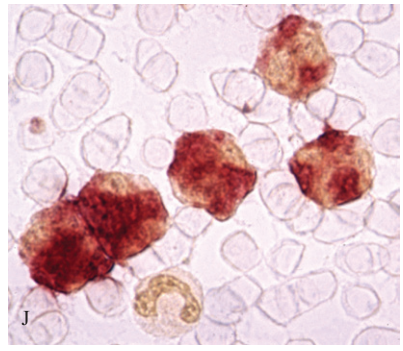
FIGURE 3.1. Morphologic appearance of various forms of lymphoma and leukemia. A. Lymphoma (lymph node, dog); B. large granular lymphoma (intestine, cat); C. plasma cell myeloma (bone marrow, dog); D. acute lymphoblastic leukemia (blood, horse); E. chronic lymphocytic leukemia (blood, horse); F. acute granulocytic leukemia (lymph node, cat); continued.

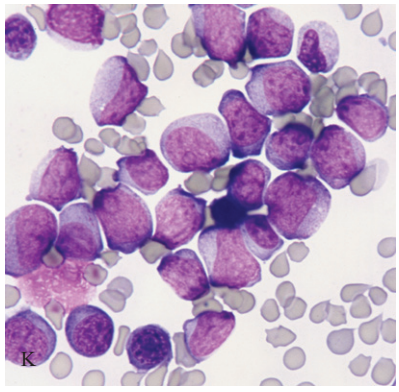
Free ebooks ==> www.Ebook777.com











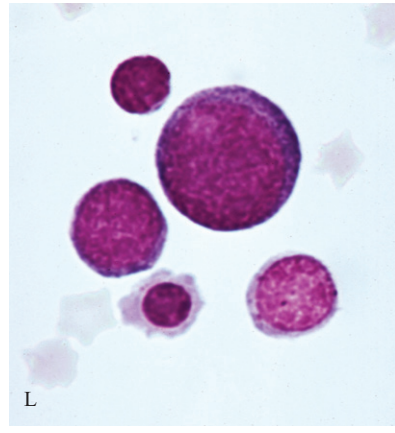


FIGURE 3.1. continued. G. acute granulocytic leukemia (blood, cat, myeloperoxidase staining); H. myelomomocytic leukemia (blood, horse); I. monocytic leukemia (blood, dog); J. monocytic leukemia (blood, dog, α-naphthyl acetate esterase staining); K. erythroleukemia (bone marrow, cat); L. erythroleukemia (blood, cat); continued.

www.Ebook777.com

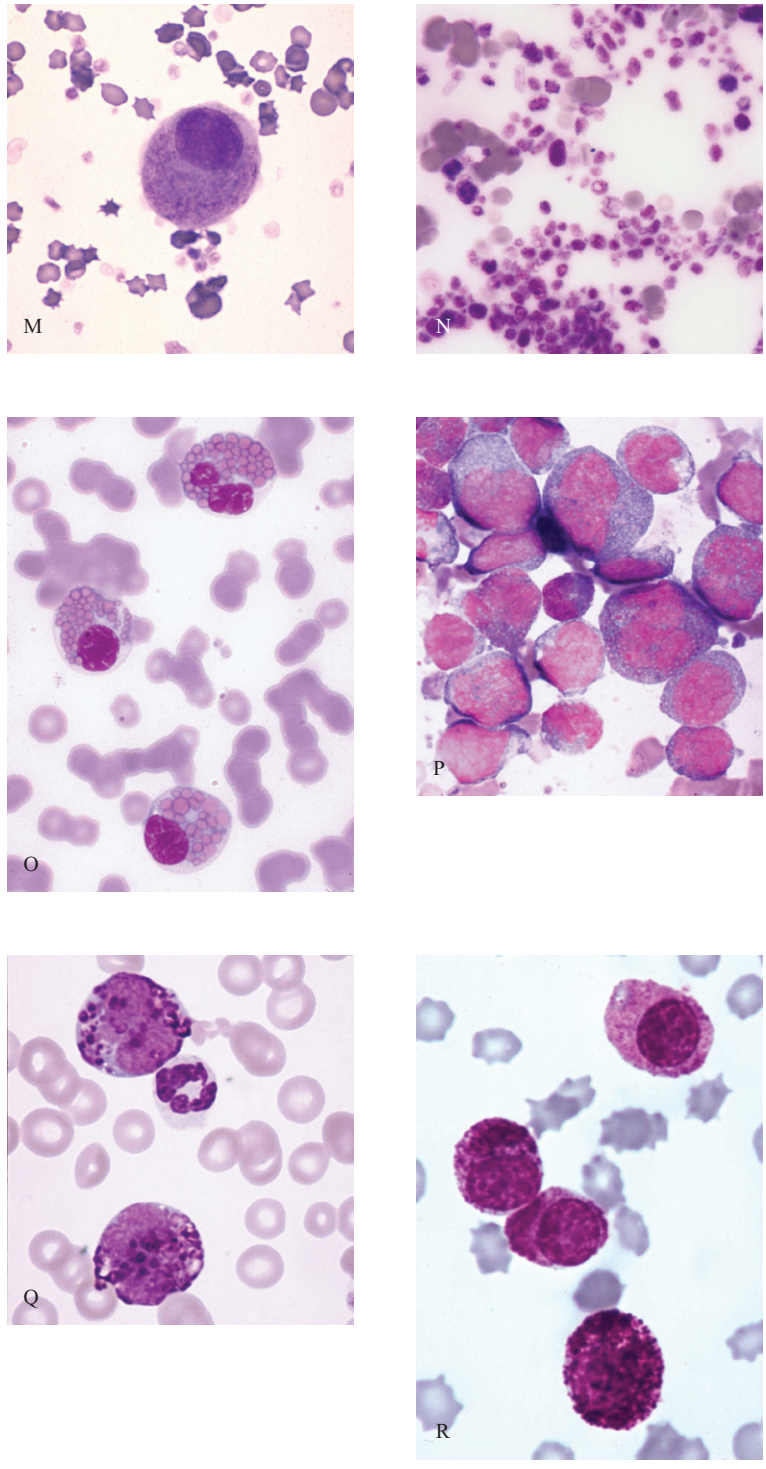


FIGURE 3.1. continued. M. megakaryocytic leukemia (megakaryocytic myelosis, blood, cat); N. essential thrombocythemia (blood, cat); O. eosinophilic leukemia (blood, horse); P. eosinophilic myeloproliferative disease (bone marrow, horse); Q. basophilic leukemia (blood, dog); R. mast cell leukemia (blood, dog).

Free ebooks ==> www.Ebook777.com

the adjective "malignant" was added, indicating that malignant lymphoma was a malignancy composed of lymphocytes.

c. Current medical terminology has evolved one step further by designating these neoplasms as "lymphoma." The adjective "malignant" was discarded from the term "malignant lymphoma" because it was redundant; lymphomas are virtually always malignant.

d. In summary, common synonyms for lymphoma include lymphosarcoma and malignant lymphoma; however, most forms of lymphoma are malignant.

2. Lymphoma may occur in very young animals, but most commonly affects middle-aged animals.

3. In advanced lymphoma, neoplastic lymphocytes frequently infiltrate the bone marrow and are present in the blood (leukemic blood profile or leukemic blood picture) even though the neoplasm originated as an extramarrow, solid tissue mass.

4. Leukemia is defined as hematopoietic neoplasia. Neoplastic cells may be present in the blood and/or bone marrow.

5. Lymphocytic leukemia may arise in the bone marrow or as a primary splenic neoplasm with a leukemic blood profile.

B. Subclassification. Lymphoma has been classified into subtypes according to anatomic distribution, histologic pattern, cellular morphology, cytochemistry, and expression of CD markers. Prognostic information regarding the clinical course of disease and response to therapy is gradually evolving as larger numbers of patients with lymphoma are evaluated prospectively by uniform criteria. Subclassification of lymphoma has been based on the following:

1. Anatomic distribution

a. Multicentric lymphoma. Multiple lymph nodes are involved in the disease. Various organs may be infiltrated by neoplastic lymphocytes, especially spleen and liver.

b. Alimentary lymphoma. The gastrointestinal tract and its regional lymph nodes are affected. Other abdominal organs may be infiltrated with neoplastic lymphocytes, but superficial lymph nodes usually are unaffected.

c. Mediastinal lymphoma. The cranial mediastinal lymph nodes or thymus are involved in the neoplastic process. This form of lymphoma occurs primarily in young animals and is uniformly of T-cell origin.

d. Cutaneous lymphoma. Lymphoma of the skin may be epitheliotropic or dermal, solitary or generalized. The draining lymph nodes frequently are enlarged.

e. Miscellaneous presentations of lymphoma. Lymphoma may originate in most organs and tissues, including the kidney, eye, and central nervous system.

2. Histologic pattern in affected lymph nodes

a. Diffuse. The diffuse pattern of lymphoma is characterized by sheets of neoplastic lymphocytes that replace the normal tissue architecture. In domestic animals, the majority of cases of lymphoma have this pattern.

b. Nodular. The nodular pattern of lymphoma is uncommon in animals. Histologic sections of affected lymph nodes have nodular aggregates of neoplastic lymphocytes, sometimes mimicking cortical follicular structures. This pattern of lymphoma has a slowly progressive clinical course of disease compared to diffuse lymphoma.

3. Morphology of the neoplastic lymphocytes

a. Attempts have been made to apply human lymphoma classification schemes to animal tumors.

b. The National Cancer Institute Working Formulation considers both the architectural pattern of the neoplastic lymph node and cellular morphology.

c. The updated Kiel classification includes architectural pattern, cellular morphology, and B or T lymphocyte origin.

d. Both the National Cancer Institute Working Formulation and Kiel schemes classify lymphomas into low-, intermediate-, or high-grade neoplasms. Both of these classification protocols predict the clinical course of canine lymphoma with reasonable accuracy.

www.Ebook777.com

e. The Revised European-American Classification of Lymphoid Neoplasms (REAL) considers cellular morphology, immunophenotype, and, in select categories, cytogenetics. This classification scheme has not been evaluated in lymphoma of domestic animals.

f. Enumeration of proliferation-associated antigens has been of limited value for prognosis in animals with lymphoma.

g. Determination of argyrophilic nucleolar organizing regions (AgNOR scores) indicates the rate of the cell cycle and, less specifically, of the number of dividing cells. The AgNOR score correlates with tumor prognosis in the dog, but not the cat.

h. Most canine and bovine lymphomas are high-grade neoplasms.

4. CD expression (immunophenotype)

a. Compared to other leukocytes, lymphocytes have few morphologic features indicating their maturity or function.

b. The microscopic appearance of lymphocytes has limited correlation with immunophenotype.

c. Monoclonal antibodies specific for markers expressed on animal lymphocytes of different maturational stages, function, and anatomic locations are increasingly available for diagnostic use.

d. Once a diagnosis of lymphoma has been established, the analysis of cell marker expression, using a panel of antibodies, may allow determination of the immunophenotype of lymphoma.

e. Marker expression of neoplastic cells can be evaluated by the following techniques:

(1) Flow cytometry on single cell suspensions in fluid medium.

(2) Immunochemistry performed on cytologic preparations.

(3) Immunochemistry performed on formalin-fixed or frozen sections of biopsy material.

f. Determining the biological origin of the neoplastic lymphocytes can improve the accuracy of prognosis and treatment of lymphoma.

g. In veterinary medicine, immunophenotyping is often a research technique that is being investigated at larger universities. It generally is not commercially available.

C. Clinical staging of lymphoma

1. The World Health Organization classification of lymphoma in animals defines five stages that have been used in determining disease prognosis. The higher the stage of disease, the poorer the clinical prognosis.

a. Stage I. Only a single lymphoid tissue or organ (excluding bone marrow) is involved.

b. Stage II. Several lymph nodes in a regional area are involved.

c. Stage III. Generalized lymph node involvement is evident.

d. Stage IV. Liver and spleen are involved (with or without stage III).

e. Stage V. In addition to the primary solid tumor, neoplastic cells are present in the blood, bone marrow, and/or other organs.

2. Each stage is further subdivided according to the absence (A) or presence (B) of systemic signs of disease.

D. Canine lymphoma

1. Anatomic and functional features

a. Multicentric lymphoma is the most common anatomic form in dogs. Neoplasia begins in the lymph nodes and progresses to involve the spleen and liver. Most superficial lymph nodes are prominently enlarged and painless on palpation.

b. Alimentary, mediastinal, and cutaneous forms of lymphoma follow in decreasing frequency. These forms of neoplasia present with infiltrative disease of the small intestine, cranial mediastinal masses, and generalized skin disease, respectively. Involvement of regional lymph nodes or adjacent organs is common.

c. The majority of multicentric lymphomas in the dog are of B-cell origin.

d. B-cell origin is more common than T-cell origin among alimentary lymphoma.

e. Mediastinal lymphoma is of T-cell origin.

Free ebooks ==> www.Ebook777.com

f. Cutaneous epitheliotropic lymphoma (mycosis fungoides) is a form of T-cell neoplasia involving the CD8+ subset of lymphocytes.

g. A subgroup of lymphomas comprised of small cells causing gradual enlargement of lymph nodes or spleen has an indolent course of disease and is most commonly of B-cell origin.

2. Laboratory features

a. Lymphopenia is more common than lymphocytosis.

b. A leukemic blood profile occurs in approximately 20% of dogs with lymphoma when presented for initial examination.

c. Mild anemia is commonly observed.

d. Mild thrombocytopenia occurs in 10% to 20% of dogs with lymphoma.

e. The neutrophil count is variable, but a mild mature neutrophilia is commonly observed.

f. Infiltration of the bone marrow by neoplastic lymphocytes can be observed in bone marrow aspirates from most dogs with marked anemia, thrombocytopenia, or a leukemic blood picture.

g. Hypercalcemia is often associated with lymphoma due to production of parathyroid hormone-related peptide (PTHrP) by neoplastic T lymphocytes. The degree of hypercalcemia may be marked and results in the activation of osteoblasts and osteoclasts. Hypercalcemia may cause renal failure via nephrocalcinosis.

h. Immune-mediated hemolytic anemia, gammopathy, or bleeding due to tumor-elaborated factors occurs infrequently.

i. Cutaneous epitheliotropic lymphoma is occasionally accompanied by large, morphologically abnormal lymphocytes in circulation (Sézary syndrome).

j. Diffuse tumor architecture and homogenous cellular morphology permit cytological diagnosis in most cases of canine lymphoma.

E. Feline lymphoma

1. The role of viral agents

a. Prior to widespread testing and vaccination, feline leukemia virus (FeLV) infection was the most common cause of feline lymphoma.

b. Currently, only 10% to 20% of lymphomas in the cat are associated with a positive FeLV antigen test.

c. In the last two decades, the average age of cats with lymphoma has increased (approximately 10 years of age), while concurrent FeLV seropositivity has decreased.

d. Cats chronically infected with the feline immunodeficiency virus (FIV) also have an increased incidence of lymphoma.

e. However, most cats with lymphoma are negative for FeLV and FIV infections by group-specific antigen testing and antibody determination on blood, respectively. In some individuals, sequestered FeLV infection subsequently may be demonstrated by polymerase chain reaction analysis of bone marrow.

2. Anatomic and functional features

a. Alimentary lymphoma is the most common form of lymphoid neoplasia in the cat, followed by multicentric, mediastinal, renal, and other extranodal lymphomas.

b. Multicentric lymphoma commonly involves the abdominal lymph nodes with or without involvement of the liver and spleen. Involvement of the superficial lymph nodes alone is infrequently observed.

c. A rare form of lymphoma, involving solitary or regional lymph nodes of the head or neck, is of atypical B-cell origin and morphologically similar to human Hodgkin's lymphoma.

d. Gastric lymphomas are of B-cell origin, while small intestinal lymphomas are of either T- or B-cell origin.

e. Large granular lymphoma of the small intestine occurs in older cats, is of T-cell origin, and associated with a leukemic blood picture.

f. Alimentary lymphoma occurs in older FeLV-negative cats.

www.Ebook777.com

g. Mediastinal lymphoma occurs in younger cats. It is of T-cell origin, and more than 80% of affected cats are FeLV seropositive.
h. The incidence of FeLV infection varies from 20% to 50% for hepatic and renal lymphoma.
i. Most spinal lymphomas are reported in young FeLV-seropositive cats.
j. Nasal lymphomas are of B-cell origin and occur in older FeLV-seronegative cats.
k. Cutaneous lymphoma in the cat is rare.
l. FIV-associated lymphomas are of B-cell origin and occur most commonly in extranodal sites.
m. Heterogeneous cellular morphology and inflammatory infiltrates preclude cytological diagnosis in most cases of alimentary and multicentric feline lymphoma.

3. Laboratory features
a. A leukemic blood profile is uncommon in cats with lymphoma, regardless of their FeLV status.
b. Changes in the hemogram are absent to mild in the majority of cats with lymphoma.
c. Mild anemia of chronic disease may occur.
d. Primary hematopoietic manifestations of FeLV infection are uncommon in cats. If present, these manifestations include severe macrocytic non-regenerative anemia, giant platelets, variable dyspoiesis of neutrophils and red cells, and/or leukemia.

F. Mustelid lymphoma

1. Anatomic and functional features
a. Lymphoma is a relatively common neoplasm in ferrets.
b. The alimentary form is most common, and occurs mostly in middle-aged to older ferrets.
c. Mediastinal lymphoma is of T-cell origin and occurs in younger ferrets, as is the case in other species.
d. Indolent as well as rapidly progressive forms of lymphoma have been reported.
e. Most lymphoma in ferrets is of T-cell origin.

2. Laboratory features
a. Typical changes in the hemogram are mild to moderate anemia.
b. Other cytopenia or a leukemic blood picture is uncommon.

G. Avian lymphoma

1. Anatomic and functional features
a. Multicentric, cutaneous and alimentary forms of lymphoma have been reported in psittacines and raptors.

2. Laboratory features
a. Hypercalcemia and gammopathy have been reported for individual cases.

H. Equine lymphoma

1. Anatomic and functional features
a. Neoplasia, other than cutaneous neoplasms, is uncommon in horses; however, lymphoma is not infrequent.
b. Multicentric lymphoma is the most frequent presentation of lymphoma in the horse. Abdominal lymph nodes are affected; concurrent intestinal and hepatic infiltration by neoplastic lymphocytes may be observed.
c. Subcutaneous, splenic, and mediastinal forms of lymphoma also occur in the horse.
d. T-cell origin is most common, but variable cell morphology and plasma cell and histiocyte infiltrates occur in both T-cell and B-cell lymphomas.
e. In T-cell-rich B-cell lymphoma, the B-cells are thought to be the neoplastic population with subsequent recruitment of mature, differentiated T-cells.
f. Cutaneous lymphoma may be of B- or T-cell origin.
g. Mediastinal lymphoma is of T-cell origin, as is the case in other species.
h. Lymphoma in the horse may be indolent. Tumor masses may wax and wane with exposure to estrogen or progesterone.

Free ebooks ==> www.Ebook777.com

2. Laboratory features
   a. Systemic inflammation with hyperfibrinogenemia, hypoalbuminemia, and hyperglobulinemia are common and accompanied clinically by ventral edema in multicentric lymphoma.
   b. Immune-mediated hemolytic anemia with a positive direct antiglobulin (Coombs') test is common and may be the presenting sign of disease.
   c. Thrombocytopenia or a leukemic blood picture occurs in 20% to 30% of horses with lymphoma.
   d. Gammopathy is frequent in multicentric lymphoma, and may be polyclonal or monoclonal.

I. Bovine lymphoma
   1. The role of bovine leukemia virus (BLV) viral infection
      a. Most lymphoma or lymphoid leukemia in mature cattle is due to infection with the bovine leukemia virus (BLV).
      b. Infection with BLV results in persistent lymphocytosis of mature, non-neoplastic B-cells in approximately 30% of infected cattle.
      c. Persistent lymphocytosis is defined as an absolute lymphocyte count greater than the reference interval for the particular age of the animal that exists for three months or more.
      d. Fewer than 5% of cows with persistent lymphocytosis develop lymphoma. Thus, persistent lymphocytosis suggests BLV infection but does not indicate the presence of lymphoma.
      e. Persistent lymphocytosis precedes the development of lymphoma by several years in the majority of BLV-infected cattle.
   2. Anatomic and functional features
      a. Multicentric (enzootic) lymphoma is the most common presentation of lymphoid neoplasia in cattle. This form of lymphoma usually affects older cattle.
      b. Multicentric lymphoma is characterized by enlargement of superficial or abdominal lymph nodes. As the disease progresses, the heart, gastrointestinal tract, uterus, kidney, and other organs become involved.
      c. The neoplastic cells are mature B lymphocytes that express major histocompatibility complex class II antigens, CD5, and surface IgM.
      d. Other sporadic forms of lymphoma are not associated with BLV infection.
      e. Sporadic multicentric lymphoma occurs in calves, sporadic mediastinal lymphoma affects yearlings, and sporadic cutaneous lymphoma occurs in young cattle.
      f. Cytologic diagnosis from tumor aspirates is suitable in most cases.
   3. Laboratory features
      a. The WBC count is highly variable, ranging from leukopenia to leukocytosis.
      b. Morphologically abnormal lymphocytes and an increased proportion of B-cells may be detected before persistent lymphocytosis is recognized.
      c. A leukemic blood profile occurs in 10% to 30% of cattle with multicentric lymphoma. The WBC count may exceed 100,000 cells/μL and lymphocytes may have abnormal morphology.
      d. Anemia is uncommon in bovine lymphoma because of the prolonged lifespan (160 days) of erythrocytes.

II. ACUTE LYMPHOCYTIC LEUKEMIA (ALL)

A. Definition and occurrence
   1. ALL arises from undifferentiated lymphocytes in the bone marrow.
   2. ALL is uncommon in all animals compared to lymphoma.
   3. ALL in FeLV-infected cats has become uncommon since the introduction of testing and vaccination.
   4. ALL may occur at any age and typically has a very short clinical course.

www.Ebook777.com

B. Laboratory features

1. Individual neoplastic lymphocytes are medium to large and have a round to slightly indented nucleus, dispersed chromatin, one to multiple nucleoli or nucleolar rings, and a thin rim of dark blue, sometimes granular cytoplasm ("blast cells").

2. Hematologic changes are variable, but severe cytopenia is typical and may affect one or more cell lines. Neoplastic lymphocytes may be restricted to the bone marrow and morphologically inapparent on blood smears (aleukemic), may circulate in scant numbers (subleukemic), or may appear in large numbers (leukemic) in the blood.

3. Cytopenia due to myelophthisis tends to be severe and progressive.

4. Cytochemical classification of the neoplastic cells has been useful to distinguish ALL from non-lymphoid malignancies.

5. Detailed classification by immunophenotype has been limited because few differentiation markers are expressed by the neoplastic lymphocytes, but most cases of ALL are of B-cell origin.

6. CD34 is an antigen expressed by undifferentiated lymphoid and myeloid precursor cells. Its presence confirms acute leukemia, but does not identify the lineage of the neoplastic cells.

7. B-, T-, and NK-cell ALL have been reported in the dog, cat, and horse.

8. Detection of clonal lymphocyte receptor gene rearrangement may be more sensitive for detection of circulating neoplastic lymphocytes than blood smear morphologic assessment.

C. Differential diagnoses

1. ALL must be distinguished from other acute leukemias, especially when there is a predominance of undifferentiated blast cells (e.g., acute myelocytic or monocytic leukemia) and chemotherapy will be attempted. Most ALL express either cytoplasmic CD79alpha or cell surface CD21 if of B-cell origin, or CD3 if of T-cell origin.

2. Advanced lymphoma may have a marked leukemic blood profile, resembling ALL. However, lymphoma is characterized by the presence of solid tissue masses that precede the development of a leukemic blood profile. In leukemia, organ infiltration and enlargement usually occur after neoplastic cells are evident in the blood.

3. In the terminal stages of disease, ALL and lymphoma may be difficult to distinguish. However, ALL is not associated with the formation of solid tissue masses.

III. CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

A. Definition and occurrence

1. CLL is a neoplasm of relatively differentiated lymphocytes that "home" to secondary lymphoid organs such as the spleen.

2. CLL typically occurs in older animals, predominantly dogs.

3. The natural clinical course of disease is protracted, often lasting months to years.

4. Splenomegaly is pronounced at diagnosis, but bone marrow becomes involved only gradually.

5. The eventual development of lymphadenopathy in CLL may mimic the clinical appearance of small cell lymphoma.

B. Laboratory features

1. The neoplastic cells resemble benign small lymphocytes. Absolute lymphocyte counts may range from 10,000 to 100,000 cells/μL.

2. Fine, pink-purple, cytoplasmic granules have been observed in the neoplastic lymphocytes of approximately 50% to 80% of dogs with CLL, and correspond to T-cell origin. These cells have erroneously been called large granular lymphocytes (LGLs). In contrast, LGLs have large granules.

3. 60% to 80% of the cases of canine CLL are of T-cell origin, specifically of the CD8+ subset.

4. B-cell CLL has been associated with monoclonal gammopathy.

Free ebooks ==> www.Ebook777.com

5. Anemia is common but rarely severe. Mild thrombocytopenia also may occur.
6. Bone marrow involvement occurs in advanced CLL, leading to insidious myelophthisis.

C. Differential diagnoses
1. Physiologic lymphocytosis of excited cats may resemble CLL but the lymphocytosis is transient and unassociated with organomegaly.
2. Infrequently, dogs with hypoadrenocorticism may exhibit moderate lymphocytosis involving small lymphocytes.
3. Reactive lymphocytosis may be seen in some infectious diseases (e.g., anaplasmosis, ehrlichiosis, Rocky Mountain spotted fever) or following vaccination. Moderate numbers of lymphocytes may appear reactive. The etiology of reactive lymphocytosis usually can be determined by further laboratory testing (e.g., rickettsial titers).

IV. LARGE GRANULAR LYMPHOCYTE (LGL) LYMPHOMA/LEUKEMIA

A. Definition and occurrence
1. In health, LGLs arise from the bone marrow and "home" to epithelial sites, primarily in the intestine.
2. Most LGLs express a T-cell receptor, CD3, and CD8, but some may be of natural killer (NK) phenotype.
3. LGL lymphomas are uncommon but comprise a distinct entity of lymphoma. LGL lymphoma has been reported in cats, horses, dogs, and birds. Neoplasms generally arise in older animals.
4. LGL lymphoma arises in the small intestine and involves abdominal lymph nodes and liver.
5. Clinical signs of anorexia and vomiting with an increased proportion of LGL cells on blood smears are usually the presenting abnormalities.
6. LGL lymphoma is a high-grade malignancy in all species. The course of disease after diagnosis is usually rapid.

B. Laboratory features
1. LGLs are variable in size and have chunky, pink to magenta cytoplasmic granules. In some neoplastic lymphocytes, the granules may cluster near the site of nuclear indentation.
2. Increased numbers of circulating LGLs usually are present and absolute counts may exceed 100,000 cells/μL.
3. Mild anemia and neutrophilia are common.
4. Development of immunoreagents to identify LGL subsets may improve the accuracy of disease diagnosis and clinical prognosis.

C. Differential diagnoses
1. T-cell origin CLL in the dog frequently consists of small lymphocytes with fine pink cytoplasmic granules, and may be mistaken for LGL lymphoma. However, T-cell origin CLL is not associated with intestinal tumors, but originates in splenic red pulp.
2. Reactive lymphocytosis involving lymphocytes with small cytoplasmic granules has been reported in dogs with chronic anaplasmosis and in birds with coccidiosis.

V. PLASMA CELL TUMORS

A. Definition and occurrence
1. Plasmacytoma and plasma cell myeloma (multiple myeloma) are clonal proliferations of B lymphocytes that often produce a homogeneous immunoglobulin or immunoglobulin fragment.
2. Plasmacytomas occur as solitary or multiple masses, most commonly involving the skin or mucous membranes. These neoplasms arise in older animals, and in dogs usually are unaccompanied by clinical signs of disease and do not elaborate a secreted immunoglobulin.

www.Ebook777.com

3. Solitary plasma cell tumors in cats have a predilection for bone or skin, and may be locally invasive.
4. Plasmacytomas may contain cytoplasmic immunoglobulin that can be demonstrated by immunochemistry, but changes are not apparent on serum electrophoretogram.
5. Plasma cell myeloma (multiple myeloma) is a clonal neoplasm of plasma cells that typically arises in the bone marrow and is often associated with monoclonal gammopathy. Osteolysis may be observed in the dog but is rare in the cat.
6. Plasma cell myelomas in animals can produce IgG, IgA, or IgM.
7. Non-secretory plasma cell myeloma is rare, but has been reported in dogs and cats.
8. In IgM-producing plasma cell myeloma, hyperviscosity is frequent due to the large size of IgM. Osteolysis is uncommon.
9. Hyperproteinemia with hyperglobulinemia and monoclonal gammopathy are considered the hallmarks of plasma cell myeloma. However, monoclonal gammopathy has been observed infrequently in some infectious diseases (e.g., feline infectious peritonitis, canine anaplasmosis).
10. Cryoglobulinemia is the production of a monoclonal immunoglobulin, such as IgM or IgG, that precipitates below body temperature.
11. Monoclonal immunoglobulins, usually of the IgM isotype, may be produced infrequently in lymphoma or CLL of B-cell origin.
12. Plasma cell myeloma is generally a neoplasm of older animals.

B. Diagnostic criteria

1. At least two of four criteria must be met to diagnose plasma cell myeloma. These criteria include the following:
   a. Radiographic evidence of osteolysis. Osteolysis is relatively common in dogs but rare in cats.
   b. Large clusters of plasma cells in the bone marrow. A greater percentage of plasma cells may be present in aspirates from osteolytic lesions. In cats, soft tissue accumulations of neoplastic plasma cells are more common than bone lesions.
   c. Monoclonal gammopathy on serum electrophoresis.
   d. Bence Jones proteinuria. Bence Jones proteins are free immunoglobulin light chains best detected by electrophoresis of concentrated urine specimens (Chapter 9).

C. Laboratory features

1. Hyperglobulinemia. This is the hallmark of plasma cell myeloma.
   a. The monoclonal immunoglobulin is recognizable as a narrow-based, discrete band or peak in the β or γ region of the serum electrophoretogram.
   b. The abnormal protein is referred to as M component or paraprotein.
   c. The paraprotein may be composed of intact immunoglobulin, light chains, or heavy chains.
   d. The immunoglobulin isotype can be identified by immunoelectrophoresis, immunofixation, or radial immunodiffusion.
   e. Synthesis of benign, active immunoglobulins may be suppressed. This functional immunodeficiency may predispose the patient to infection because the monoclonal antibody does not provide broad protection against pathogens.
2. Bence Jones proteinuria
   a. Free immunoglobulin light chains (Bence Jones proteins) are smaller than albumin, readily pass the glomerulus, and are present at greater concentration in urine than in serum. Thus, their detection in urine is of diagnostic importance. “Light chain disease” does not result in monoclonal gammopathy on serum protein electrophoresis.
   b. Intact immunoglobulin molecules do not pass the glomerular filter, unless glomerular damage is present.
   c. Light chains do not react with the protein indicator pad on urine dipsticks; thus, alternate methods are used to detect this protein.

Free ebooks ==> www.Ebook777.com

d. Electrophoresis of concentrated urine is the preferred method to detect Bence Jones protein; heat precipitation of urine light chains is insensitive and non-specific.
e. More than 50% of dogs and cats with plasma cell myeloma may have light chain proteinuria.

3. Bone marrow and hematology
a. Neoplastic plasma cells in the bone marrow frequently are indistinguishable from benign plasma cells by light microscopy.
b. Clusters of plasma cells in the bone marrow are observed in the majority of multiple myelomas, but may be missed in a single bone marrow aspirate or core biopsy.
c. Greater than 15% to 20% plasma cells in the bone marrow is highly suggestive of plasma cell myeloma. Up to 5% plasma cells, dispersed as single cells, may be found in the bone marrow of healthy or diseased animals that do not have plasma cell myeloma.
d. Plasma cell leukemia is rare. In plasma cell myeloma, the neoplastic plasma cells rarely circulate in numbers detectable on blood smear review.
e. Anemia is consistently present and results from myelophthisis, relative blood dilution due to increased plasma oncotic pressure, or shortened erythrocyte lifespan due to coating of erythrocytes by the paraprotein with subsequent phagocytosis by splenic macrophages.
f. Thrombocytopenia and neutropenia develop with progressive myelophthisis.

4. Hemostasis
a. Platelet function may be impaired due to binding of the paraprotein to platelets (e.g., decreased aggregation).
b. Complexing of the paraprotein to coagulation factors reduces clotting.
c. IgM (MW = 900,000 daltons) and IgA dimers may cause increased viscosity of blood, resulting in tissue ischemia and hemorrhage.
d. Thus, bleeding may be observed in cases of plasma cell myeloma despite a normal platelet count.

5. Other laboratory features
a. Moderate hypercalcemia is common. Myeloma cells produce osteoclast-activating factors resulting in resorption of bone.
b. Renal disease develops from nephrocalcinosis secondary to chronic hypercalcemia, hypoxic damage from hyperviscosity, renal toxicity of light chains, neoplastic cell infiltration of the kidneys, and/or primary amyloid deposition (the latter occurs mainly in humans).
c. Hyperviscosity relates to the size, polymerization, and amount of paraprotein. It is a common feature of IgM myeloma, and less frequently observed in IgA and IgG myelomas.
d. Compensatory hypoalbuminemia may occur to offset the hyperglobulinemia-induced increase in plasma oncotic pressure.

MYELOPROLIFERATIVE DISORDERS

Myeloproliferative disorder is a general term for all leukemias that originate in the bone marrow and involve cells other than lymphocytes. Leukemia is a clonal disorder wherein a single neoplastic cell gives rise to a homogeneous progeny. The neoplastic progeny cells may be relatively undifferentiated (acute leukemia) or resemble more mature cells that are normally encountered in the blood (chronic leukemia) (Figure 3.1). Leukemia cells have multiple genetic lesions that result in particular phenotypes according to expression or repression of stimulating factors or receptors for specific cell lineages. Normal hematopoietic cells are displaced by the neoplastic population, resulting in myelophthisic disease. Acute leukemia has a rapid and fatal clinical course without treatment. In contrast, untreated chronic leukemia has a more gradual onset and longer clinical course prior to death. Anemia, thrombocytopenia, and neutropenia are greater clinical complications in chronic leukemia because of the longer course of disease.

www.Ebook777.com

I. GENERAL CHARACTERISTICS

A. A leukemic blood profile is usually present.

B. Classification into acute or chronic leukemia is based on the percentage of undifferentiated blast cells in the blood or bone marrow.

C. This classification corresponds to the clinical course of the disease. Acute leukemia is rapidly progressive, whereas chronic leukemia is more slowly progressive.

D. The neoplastic cells infiltrate organs in a pattern similar to the distribution of bone-marrow-derived monocytes and macrophages. Infiltrative disease commonly involves the splenic red pulp, sinusoidal and subcapsular areas of lymph nodes, sinusoidal and portal regions of the liver, and vascular areas of other organs.

E. Leukemic cells generally do not form discrete masses. An exception is the formation of green tissue masses, called chloromas, that may rarely be observed in granulocytic leukemia. Similar masses associated with myeloid leukemia in birds have been called myelocytomas.

F. In acute leukemia, hematopoietic bone marrow is partially or completely replaced by neoplastic cells, which may result in hypo- or hypercellularity.

G. Bone marrow hypercellularity, gradually developing hepatosplenomegaly, and mild lymphadenopathy are characteristic of chronic leukemia.

H. Myeloproliferative diseases are much less common than lymphoproliferative diseases.

II. ACUTE MYELOID LEUKEMIA (AML)

A. Classification

1. Acute myeloid leukemia in humans is categorized according to the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues, which considers morphologic, immunophenotypic, and genetic features to arrive at distinct entities that have clinical and prognostic similarity. This classification scheme has been adapted for acute leukemias in dogs and cats, with predominant reliance on morphologic features.

2. Acute myeloid leukemia in dogs and cats can be classified into most of the human subgroups.

3. A diagnosis of acute leukemia is made when more than 30% of the nucleated cells in the bone marrow are blast cells and the total nonerythroid cells comprise more than 50% of the nucleated cell population.

4. Blast cells of different cell lineages have few distinguishing morphologic features in Romanowsky-stained blood and bone marrow smears. Therefore, panels of cytochemical or immunochemical reactions frequently are necessary to identify specific lineage of the blast cells.

5. Transmission electron microscopic evaluation may assist in classification of leukemia cells according to cytoplasmic features.

6. Acute leukemias may have profound genetic abnormalities and are rapidly fatal.

7. Current technology does not permit classification of all acute leukemias in animals.

B. Subgroups

1. Acute undifferentiated leukemia (AUL). This form of leukemia is characterized by large numbers of blast cells whose lineage cannot be determined. In the dog, these cells can be labeled with an anti-CD34 antibody, which identifies undifferentiated lymphocytic and myelocytic precursor cells in the bone marrow.

2. Acute myeloblastic (granulocytic, neutrophilic) leukemia

a. Two subtypes may be recognized in animals.

Free ebooks ==> www.Ebook777.com

(1) Myeloblastic leukemia without maturation. In this form of AML (M1), more than 90% of the nonerythroid cells in the bone marrow are myeloblasts.
(2) Myeloblastic leukemia with maturation. In this form of AML (M2), 30% to 90% of the nonerythroid cells are myeloblasts. Basophilic or eosinophilic differentiation may be present.

b. Laboratory features include the following:
(1) A leukemic blood profile with a variable number of myeloblasts. Neutrophilic blasts are myeloperoxidase, Sudan Black B, and chloroacetate esterase positive. In the dog and cat, neoplastic neutrophil precursors may exhibit alkaline phosphatase staining, whereas normal neutrophils are negative (alkaline phosphatase activity is a marker of immaturity in many cell lines, including lymphocytes).
(2) Myeloperoxidase activity may be detected by automated hematology analyzers.
(3) The leukemic blasts express CD34 and the pan-leukocyte marker CD45, and may express neutrophil specific antigen (NSA).
(4) Total leukocyte counts vary from leukopenia to leukocytosis. A bone marrow aspirate is essential for diagnosis of AML in cases with leukopenia (neutropenia) and few neoplastic cells in the blood. Bone marrow should contain more than 30% blast cells.
(5) Nonregenerative anemia and thrombocytopenia result from myelophthisis. The anemia may be severe.

3. Promyelocytic leukemia (M3)
a. The neoplastic cells have a few azurophilic, cytoplasmic granules.
b. Cytochemical reactivity is consistent with neutrophil lineage.
c. This subtype of AML has been reported only in pigs.

4. Myelomonocytic leukemia (M4)
a. This subtype is the most common form of AML of dogs, cats, and horses.
b. Both neutrophilic and monocytic cell lines are affected, demonstrating their shared bipotential precursor cell (CFU-GM; see Figure 1.2 in Chapter 1).
c. Laboratory features
(1) Myeloblasts and monoblasts in the bone marrow comprise more than 30% of all nucleated cells.
(2) Neutrophilic blasts are identified by cytochemical reactivity for myeloperoxidase, Sudan Black B, and chloracetate esterase.
(3) Monocytic blasts are identified by reactivity for α-naphthyl actetate esterase (non-specific esterase) activity that can be inhibited by sodium fluoride treatment. These cells also have reactivity for α-naphthyl butyrate esterase (specific esterase) activity.
(4) Immunochemical detection of CD14 expression or reactivity with antibody MAC387 (calprotectin) is supportive of monoblastic or monocytic origin.
(5) The percentage of neutrophils and monocytes may change during the course of disease; either cell type may predominate.
(6) Of the nucleated cells in the bone marrow, more than 20% are maturing granulocytes and more than 20% are maturing monocytes.

5. Monocytic leukemia (M5)
a. Cells of the monocytic lineage, as identified by cytochemistry and/or CD14 or calprotectin immunostaining, comprise more than 80% of the nonerythroid nucleated cells in the bone marrow.
b. Marked monocytosis is present in the blood. The monocytes may be immature or appear abnormal.
c. Mild anemia and thrombocytopenia are common. The mild degree of anemia, even with marked leukocytosis, may be related to lack of a monocyte storage pool in the bone marrow.

6. Erythroleukemia (M6)
a. Erythroleukemia is characterized by the co-production of erythroblasts and myeloblasts. This form of leukemia has a dual cell lineage.

www.Ebook777.com

b. More than 50% of the nucleated bone marrow cells are erythroid precursors, while more than 30% of the non-erythroid cells are myeloblasts. Dyserythropoiesis is common.
c. Erythroid precursors are recognized by dark blue cytoplasmic staining. Neither cytochemical staining nor immunochemical markers are available for erythroblast identification.
d. Erythroleukemia has only been reported in cats and poultry, and is often associated with FeLV infection in cats.
e. Erythemic myelosis is an older term used for FeLV-associated erythroleukemia in cats.
f. Laboratory features
(1) This leukemia is characterized by severe anemia, marked metarubricytosis with variable maturation stages of erythroid precursor cells in the blood, and an absence of polychromasia or reticulocytosis.
(2) Thus, this condition is a severe nonregenerative anemia and leukemia.
(3) Acute and chronic forms of erythemic myelosis are recognized. The acute form has more rubriblasts and prorubricytes. The chronic form has a predominance of rubricytes and metarubricytes; erythroblasts are infrequently observed. This condition may transition into acute undifferentiated leukemia.
(4) This manifestation of FeLV infection has become less common since the advent of FeLV testing and vaccination.

7. Megakaryocytic leukemia (megakaryocytic myelosis, M7)
a. More than 30% of the nucleated bone marrow cells are megakaryoblasts. These cells may be difficult to recognize by morphology alone.
b. If necessary, megakaryoblasts may be identified by cytochemistry by identifying acetylcholine esterase reactivity. Alternatively, immunoreactivity for von Willebrand factor or glycoprotein IIb-IIIa (CD41–61) may be used for cellular identification. Concurrent expression of CD34 identifies cells as megakaryoblasts.
c. Myelofibrosis and inability to aspirate bone marrow is a feature of megakaryocytic leukemia.

III. MYELODYSPLASTIC SYNDROME (MDS)

A. Definition
1. MDS is a clonal hematopoietic disorder that commonly evolves into overt acute leukemia. Thus, MDS is a preleukemic condition.
2. The presence of fewer than 30% blast cells in the bone marrow, dyserythropoiesis (abnormal erythrocyte morphology), and cytopenia affecting more than one hematopoietic cell line define MDS.
3. MDS has been reported most commonly in cats and infrequently in dogs and horses.
4. Cats infected with FeLV may develop MDS.

B. Laboratory features
1. Cytopenia affecting more than one hematopoietic cell line may cause non-regenerative anemia, neutropenia, and thrombocytopenia in any combination.
2. Typical hematologic changes in MDS include macrocytosis without polychromasia, metarubricytosis, abnormally maturing rubricytes, giant platelets, hypersegmented and giant neutrophils, circulating myeloblasts, and abnormal eosinophils.
3. Bone marrow aspirates and core biopsies are often hypercellular. Dysplastic changes, in addition to those observed in the blood, may include megakaryocytic nuclear hypo- or hyperlobulation, dwarf megakaryocytes with round nuclei, megakaryocyte clustering, megakaryocyte and rubricyte nuclear:cytoplasmic asynchrony, and abnormal granulation of granulocytic precursors.
4. Many animals with MDS progress to a type of AML, although the transitional period is of variable length.

Free ebooks ==> www.Ebook777.com

IV. MYELOPROLIFERATIVE NEOPLASMS (MPN)

A. Definition

1. MPN encompasses clonal neoplastic proliferations of mature and less mature granulocytes, erythrocytes, and platelets with gradual disease progression.

2. Common clinical features are bone marrow hypercellularity, splenomegaly, and moderate to marked leukocytosis, erythrocytosis, or thrombocytosis.

3. MPN is generally a disease of older animals.

4. Transformation into acute myeloid leukemia may occur along with a change to a predominance of undifferentiated cells in the blood (blast crisis).

B. Subgroups

1. Chronic granulocytic leukemia (CGL)

a. CGL is characterized by the proliferation of relatively mature neutrophils.

b. CGL has been described infrequently in dogs, cats and horses.

c. Hematologic changes include a moderate to severe neutrophilia with a disorderly left shift. Neutrophilic segmenters, bands, and metamyelocytes predominate, but myelocytes, promyelocytes, and myeloblasts invariably are present.

d. Moderately differentiated granulocytic cells predominate in the bone marrow with myeloid to erythroid ratios ranging from 5:1 to more than 20:1.

e. The degree of maturation of the neutrophilic precursors distinguishes CGL from AML in bone marrow aspirates; AML has a predominance of myeloblasts.

f. Mild anemia is common, and a variable degree of thrombocytopenia may be present.

g. CGL must be differentiated from leukemoid inflammatory responses, which are characterized by a marked neutrophilia and severe left shift. A diagnosis of CGL is made by excluding the possibility of inflammation and infection (e.g., pyometra, abscesses) and observing abnormal neutrophil morphology. The pattern of tissue and organ infiltration in CGL is not characteristic of an inflammatory response.

h. CGL also must be differentiated from paraneoplastic syndromes associated with the aberrant production of granulocyte- or granulocyte-macrophage colony-stimulating factor (G-CSF or GM-CSF, respectively) by neoplasms such as renal carcinoma, rectal adenoma, fibrosarcoma, and other tumors. These paraneoplastic syndromes may be associated with neutrophil counts in excess of 100,000 to 200,000 cells/μL.

2. Chronic eosinophilic leukemia

a. Marked eosinophilia in blood and bone marrow is accompanied by variable eosinophil maturation.

b. This form of leukemia is rare, but has been observed in the cat.

c. Chronic eosinophilic leukemia must be differentiated from hypereosinophilic syndrome in cats and Rottweiler dogs, where mild to moderate blood eosinophilia is accompanied by nonspecific tissue infiltration by eosinophils.

3. Chronic basophilic leukemia

a. Basophils predominate in the blood and bone marrow.

b. This form of leukemia is rare, but has been described in the dog and cat.

c. Basophils are distinguished from mast cells by the presence of segmented nuclei and less intense granulation. Nuclear hyposegmentaion of neoplastic basophils may make these cells more difficult to differentiate from mast cells. Basophil granules have a more chunky appearance as compared to the finer granules of mast cells.

4. Polycythemia vera (primary erythrocytosis, essential erythrocytosis)

a. This form of hematologic neoplasia represents proliferation of "mature" erythrocytes independent of erythropoietin stimulation.

www.Ebook777.com

b. Animals with polycythemia vera have an elevated hematocrit (60% to 80%) despite adequate hydration and oxygenation.
c. Erythrocytes on the stained blood film are morphologically normal.
d. Polycythemia vera has been described in cats, dogs, cattle, and horses.
e. Polycythemia vera must be differentiated from absolute polycythemia that infrequently occurs due to increased erythropoietin production by kidneys with cysts or certain neoplasms (e.g., renal carcinoma).
f. In polycythemia vera, erythropoietin concentrations are at the low end of the reference interval or decreased.
g. Hemorrhagic gastroenteritis in dogs may be associated with marked polycythemia (PCV = 60% to 80%); however, clinical signs make the diagnosis obvious.

5. Essential thrombocythemia
a. This form of leukemia is rare but has been described in dogs and cats.
b. Platelet counts in the blood typically exceed $1 \times 10^6/\mu L$.
c. The platelets may be morphologically abnormal with giant platelets and intense granulation.
d. Megakaryocytes are numerous in bone marrow aspirates and core biopsies.

6. Chronic myelomonocytic leukemia
a. This form of leukemia involves excessive production of neutrophils and monocytes.
b. Percentages of neutrophils and monocytes are variable and may change throughout the course of disease.
c. The neutrophils and monocytes appear mature or well differentiated on the stained blood smear.
d. The degree of anemia often is less severe that in chronic granulocytic leukemia but more severe than in chronic monocytic leukemia.
e. Hepatosplenomegaly is common.

7. Chronic monocytic leukemia
a. This form of leukemia is characterized by the excessive production of monocytes.
b. Extreme leukocytosis may be present with monocyte counts that approach 800,000 cells/μL.
c. The monocytes appear mature or well differentiated on the stained blood smear.
d. The degree of anemia often is mild despite extreme leukocytosis. This may be due to the lack of a bone marrow maturation and storage pool for monocytes (Chapter 2).
e. Hepatosplenomegaly is common.

V. MAST CELL LEUKEMIA

1. This form of leukemia is characterized by circulating mast cells that either appear morphologically normal or have variable granulation, cell size, and nuclear size.
2. Mast cell leukemia may occur as a primary hematopoietic neoplasm, which arises in the absence of cutaneous neoplasms in dogs or gastrointestinal neoplasms in cats.
3. In cats, splenic mast cell tumors often have systemic mastocytosis with a leukemic blood profile.
4. Mast cell leukemia is rare. It must be differentiated from solid mast cell tumors with a leukemic blood profile, as well as from benign mastocytemia that may accompany parvoviral enteritis, gastric torsion, pericarditis, pleuritis, peritonitis, aspiration pneumonia, and pancreatic necrosis.
5. Gastrointestinal illness in mast cell leukemia is common due to release of vasoactive mediators such as histamine.

VI. MYELOID-DERIVED MACROPHAGE AND DENDRITIC CELL NEOPLASMS

A. Definition
1. Histiocytes and dendritic cells derived from bone marrow precursors may give rise to solid tumors affecting skeletal muscle, spleen, skin, lung, bone marrow, and other sites.

Free ebooks ==> www.Ebook777.com

2. Clinical, morphological, and immunophenotypic characteristics allow distinction of histiocytic from dendritic cell origin.
3. Tumors are most commonly diagnosed in dogs, and there is a breed predisposition for Bernese Mountain, Rottweiler, and flat-coated Retriever dogs.
4. Chronic leukemia of dendritic cells has been described in dogs.

B. Subgroups

1. Histiocytic sarcoma of interstitial dendritic cell origin comprises solid masses in periarticular regions, lung, or spleen of dogs. Localized and disseminated forms occur. Disseminated histiocytic sarcoma was previously termed "malignant histiocytosis."
2. Histiocytic sarcoma cells are characterized by expression of CD1a, CD11c, and major histocompatiblity class II antigens, characteristic of dendritic cells
3. The hemophagocytic variant of histiocytic sarcoma is of bone marrow macrophage origin. These neoplasms occur in spleen, bone marrow, and liver, and are of a diffuse and infiltrative nature, rarely forming solid masses.
4. Hemophagocytic histiocytic sarcoma is characterized by phagocytosis of erythrocytes and other blood cells. Affected dogs have anemia, thrombocytopenia, hypoalbuminemia, and hypocholesterolemia.
5. The neoplasm is characterized by expression of CD11d with absence of CD1 and CD11c, consistent with macrophages.
6. Dendritic cell leukemia is rare. In a single case report there were no cytopenias but marked leukemia with tissue infiltrates.
7. Chronic histiocyte proliferations affecting the skin have been described in cats.

REFERENCES

Affolter VK, Moore PF: 2006. Feline progressive histiocytosis. *Vet Pathol*. 43:646–655.

Ammersbach M, Delay J, Caswell JL, et al: 2008. Laboratory findings, histopathology, and immunophenotype of lymphoma in domestic ferrets. *Vet Pathol* 45:663–673.

Allison RW, Brunker JD, Breshears MA, et al: Dendritic cell leukemia in a Golden Retriever. *Vet Clin Pathol* 37:190–197, 2008.

Beatty JA, Lawrence CE, Callanan JJ, et al: 1998. Feline immunodeficiency virus (FIV)-associated lymphoma: a potential role for immune dysfunction in tumourigenesis. *Vet Immunol Immunopathol* 65:309–322.

Bienzle D, Silverstein DC, Chaffin K: 2000. Multiple myeloma in cats: variable presentation with different immunoglobulin isotypes in two cats. *Vet Pathol* 37:364–369.

Boone LI, Knauer KW, Rapp SW, et al: 1998. Use of human recombinant erythropoietin and prednisone for treatment of myelodysplastic syndrome with erythroid predominance in a dog. *J Am Vet Med Assoc* 213:999–1001.

Breuer W, Colbatzky F, Platz S, et al: 1993. Immunoglobulin-producing tumours in dogs and cats. *J Comp Pathol* 109:203–216.

Caniatti M, Roccabianca P, Scanziani E, et al: 1996. Canine lymphoma: immunocytochemical analysis of fine-needle aspiration biopsy. *Vet Pathol* 33:204–212.

Cesari A, Bettini G, Vezzali E: 2009. Feline intestinal T-cell lymphoma: assessment of morphologic and kinetic features in 30 cases. *J Vet Diagn Invest* 21:277–279.

www.Ebook777.com

Colbatzky F, Hermanns W: 1993. Acute megakaryoblastic leukemia in one cat and two dogs. *Vet Pathol* 30:186–194.

Dascanio JJ, Zhang CH, Antczak DF, et al: 1992. Differentiation of chronic lymphocytic leukemia in the horse. A report of two cases. *J Vet Intern Med* 6:225–229.

Darbes J, Majzoub M, Breuer W, et al: 1998. Large granular lymphocyte leukemia/lymphoma in six cats. *Vet Pathol* 35:370–379.

Day MJ, Henderson SM, Belshaw Z, et al: 2004. An immunohistochemical investigation of 18 cases of feline nasal lymphoma. *J Comp Pathol.* 130:152–161.

de Bruijn CM, Veenman JN, Rutten VP, et al: 2007. Clinical, histopathological and immunophenotypical findings in five horses with cutaneous malignant lymphoma. *Res Vet Sci* 83:63–72.

de Wit M, Schoemaker NJ, Kik MJ, et al: 2003. Hypercalcemia in two Amazon parrots with malignant lymphoma. *Avian Dis* 47:223–228.

Erdman SE, Brown SA, Kawasaki TA, et al: 1996. Clinical and pathologic findings in ferrets with lymphoma: 60 cases (1982–1994). *J Am Vet Med Assoc* 208:1285–1289.

Fine DM, Tvedten HW: 1999. Chronic granulocytic leukemia in a dog. *J Am Vet Med Assoc* 214:1809–1812.

Fournel-Fleury C, Ponce F, Felman P, et al: 2002. Canine T-cell lymphomas: a morphological, immunological, and clinical study of 46 new cases. *Vet Pathol* 39:92–109.

Gabor LJ, Jackson ML, Trask B, et al: 2001. Feline leukaemia virus status of Australian cats with lymphosarcoma. *Aust Vet J* 79:476–481.

Gabor LJ, Canfield PJ, Malik R: 1999. Immunophenotypic and histological characterisation of 109 cases of feline lymphosarcoma. *Aust Vet J* 77:436–441.

Gavazza A, Lubas G, Valori E, et al: 2008. Retrospective survey of malignant lymphoma cases in the dog: clinical, therapeutical and prognostic features. *Vet Res Commun* 32 (Suppl 1):S291–S293.

Gregory CR, Latimer KS, Mahaffey EA, et al: 1996. Lymphoma and leukemic blood picture in an emu (*Dromaius novae hollandiae*). *Vet Clin Pathol* 25:136–139.

Grindem CB, Roberts MC, McEntee MF, et al: 1989. Large granular lymphocyte tumor in a horse. *Vet Pathol* 26:86–88.

Guglielmino R, Canese MG, Miniscalco B, et al: 1997. Comparison of clinical, morphological, immunophenotypical and cytochemical characteristics of LGL leukemia/lymphoma in dog, cat and human. *Eur J Histochem* Suppl 2:23–24.

Hammer AS, Williams B, Dietz HH, et al: 2007. High-throughput immunophenotyping of 43 ferret lymphomas using tissue microarray technology. *Vet Pathol* 44:196–203.

Haney SM, Beaver L, Turrel J, et al: 2009. Survival analysis of 97 cats with nasal lymphoma: a multi-institutional retrospective study (1986–2006). *J Vet Intern Med* 23:287–294.

Hisasue M, Nishimura T, Neo S, et al: 2008. A dog with acute myelomonocytic leukemia. *J Vet Med Sci* 70:619–621.

Jain NC, Blue JT, Grindem CB, et al: 1991. Proposed criteria for classification of acute myeloid leukemia in dogs and cats. *Vet Clin Pathol* 20:63–82.

Kelley LC, Mahaffey EA: 1998. Equine malignant lymphomas: morphologic and immunohistochemical classification. *Vet Pathol* 35:241–252.

Free ebooks ==> www.Ebook777.com

Khanna C, Bienzle D: 1994. Polycythemia vera in a cat. *J Am Animal Hosp Assoc* 30:45–49.

Kiselow MA, Rassnick KM, McDonough SP, et al: 2008. Outcome of cats with low-grade lymphocytic lymphoma: 41 cases (1995–2005). *J Am Vet Med Assoc* 232:405–410.

Kiupel M, Teske E, Bostock D: 1999. Prognostic factors for treated canine malignant lymphoma. *Vet Pathol* 36:292–300.

Krick EL, Little L, Patel R, et al: 2008. Description of clinical and pathological findings, treatment and outcome of feline large granular lymphocyte lymphoma (1996–2004). *Vet Comp Oncol* 6:102–110.

Lane SB, Kornegay JN, Duncan JR, et al: 1994. Feline spinal lymphosarcoma: a retrospective evaluation of 23 cats. *J Vet Intern Med* 8:99–104.

Latimer KS, Rakich PM: 1996. Sézary syndrome in a dog. *Comp Haematol Int* 6:115–119.

Latimer KS, White SL: 1996. Acute monocytic leukemia (M5a) in a horse. *Comp Haematol Int* 6:111–114.

Lennox TJ, Wilson JH, Hayden DW, et al: 2000. Hepatoblastoma with erythrocytosis in a young female horse. *J Am Vet Med Assoc* 216:718–721.

Lester GD, Alleman AR, Raskin RE, et al: 1993. Pancytopenia secondary to lymphoid leukemia in three horses. *J Vet Intern Med* 7:360–363.

Little L, Patel R, Goldschmidt M: 2007. Nasal and nasopharyngeal lymphoma in cats: 50 cases (1989–2005). *Vet Pathol* 44:885–892.

McManus PM: 2005. Classification of myeloid neoplasms: a comparative review. *Vet Clin Pathol* 34:189–212.

Majzoub M, Breuer W, Platz SJ, et al: 2003. Histopathologic and immunophenotypic characterization of extramedullary plasmacytomas in nine cats. *Vet Pathol* 40:249–253.

Malka S, Crabbs T, Mitchell EB, et al: 2008. Disseminated lymphoma of presumptive T-cell origin in a great horned owl (*Bubo virginianus*). *J Avian Med Surg* 22:226–233.

Marks SL, Moore PF, Taylor DW, et al: 1995. Nonsecretory multiple myeloma in a dog: immunohistologic and ultrastructural observations. *J Vet Intern Med.* 9:50–54.

Mears EA, Raskin RE, Legendre AM: 1997. Basophilic leukemia in a dog. *J Vet Intern Med* 11:92–94.

Mellanby RJ, Craig R, Evans H, et al: 2006. Plasma concentrations of parathyroid hormone-related protein in dogs with potential disorders of calcium metabolism. *Vet Rec* 159:833–838.

Mellor PJ, Polton GA, Brearley M, et al: 2007. Solitary plasmacytoma of bone in two successfully treated cats. *J Feline Med Surg* 9:72–77.

Mellor PJ, Haugland S, Smith KC, et al: 2008. Histopathologic, immunohistochemical, and cytologic analysis of feline myeloma-related disorders: Further evidence for primary extramedullary development in the cat. *Vet Pathol* 45:159–173.

Messinger JS, Windham WR, Ward CR: 2009. Ionized hypercalcemia in dogs: A retrospective study of 109 cases (1998–2003). *J Vet Intern Med* 23:514–519.

Meyer J, Delay J, Bienzle D: 2006. Clinical, laboratory, and histopathologic features of equine lymphoma. *Vet Pathol* 43:914–924.

Momoi Y, Nagase M, Okamoto Y, et al: 1993. Rearrangements of immunoglobulin and T-cell receptor genes in canine lymphoma/leukemia cells. *J Vet Med Sci* 55:775–780.

www.Ebook777.com

Moore PF, Olivry T, Naydan D: 1994. Canine cutaneous epitheliotropic lymphoma (mycosis fungoides) is a proliferative disorder of CD8+ T cells. *Am J Pathol* 144:421–429.

Moore PF, Affolter VK, Vernau W: 2006. Canine hemophagocytic histiocytic sarcoma: a proliferative disorder of CD11d+ macrophages. *Vet Pathol* 43:632–645.

Mylonakis ME, Petanides TA, Valli VE, et al: Acute myelomonocytic leukaemia with short-term spontaneous remission in a cat. *Aust Vet J* 86:224–228, 2009.

Patel RT, Caceres A, French AF, et al: 2005. Multiple myeloma in 16 cats: a retrospective study. *Vet Clin Pathol* 34:341–352.

Peterson EN, Meininger AC: 1997. Immunoglobulin A and immunoglobulin G biclonal gammopathy in a dog with multiple myeloma. *J Am Anim Hosp Assoc* 33:45–47.

Pohlman LM, Higginbotham ML, Welles EG, et al: 2009. Immunophenotypic and histologic classification of 50 cases of feline gastrointestinal lymphoma. *Vet Pathol* 46:259–268.

Puette M, Latimer KS: 1997. Acute granulocytic leukemia in a slaughter goat. *J Vet Diagn Invest* 9:318–319.

Raskin RE, Krehbiel JD: 1989. Prevalence of leukemic blood and bone marrow in dogs with multicentric lymphoma. *J Am Vet Med Assoc* 194:1427–1429.

Rendle DI, Durham AE, Thompson JC, et al: 2007. Clinical, immunophenotypic and functional characterisation of T-cell leukaemia in six horses. *Equine Vet J* 39:522–528.

Roccabianca P, Vernau W, Caniatti M, et al: 2006. Feline large granular lymphocyte (LGL) lymphoma with secondary leukemia: primary intestinal origin with predominance of a CD3/CD8(alpha)(alpha) phenotype. *Vet Pathol* 43:15–28.

Scott MA, Stockham SL: 2000. Basophils and mast cells. *In:* Feldman BF, Zinkl JG, Jain NC (eds): *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 308–317.

Shimoda T, Shiranaga N, Mashita T, et al: 2000. Chronic myelomonocytic leukemia in a cat. *J Vet Med Sci* 62:195–197.

Souza MJ, Newman SJ, Greenacre CB, et al: 2008. Diffuse intestinal T-cell lymphosarcoma in a yellow-naped Amazon parrot (*Amazona ochrocephala auropalliata*). *J Vet Diagn Invest* 20:656–660.

Tasca S, Carli E, Caldin M, et al. 2009. Hematologic abnormalities and flow cytometric immunophenotyping results in dogs with hematopoietic neoplasia: 210 cases (2002–2006). *Vet Clin Pathol* 38:2–12.

Teske E, van Heerde P, Rutteman GR, et al: 1995. Prognostic factors for treatment of malignant lymphoma in dogs. *J Am Vet Med Assoc* 205:1722–1728.

Teske E, Wisman P, Moore PF, et al: 1994. Histologic classification and immunophenotyping of canine non-Hodgkin's lymphomas: unexpected high frequency of T cell lymphomas with B-cell morphology. *Exp Hematol* 22:1179–1187.

Vail DM, Moore AS, Ogilvie GK, et al: 1998. Feline lymphoma (145 cases): proliferation indices, cluster of differentiation 3 immunoreactivity, and their association with prognosis in 90 cats. *J Vet Intern Med* 12:349–354.

Valli VE, Jacobs RM, Norris A, et al: 2000. The histologic classification of 602 cases of feline lymphoproliferative disease using the National Cancer Institute Working Formulation. *J Vet Diagn Invest*. 12:295–306.

Free ebooks ==> www.Ebook777.com

Valli VE, Vernau W, de Lorimier LP, et al: 2006. Canine indolent nodular lymphoma. *Vet Pathol* 43:241–256.

Vernau W, Jacobs RM, Valli VE, et al: 1997. The immunophenotypic characterization of bovine lymphomas. *Vet Pathol* 34:222–225.

Vernau W, Jacobs RM, Davies C, et al: 1998. Morphometric analysis of bovine lymphomas classified according to the National Cancer Institute Working Formulation. *J Comp Pathol* 118:281–289.

Vernau W, Moore PF: 1999. An immunophenotypic study of canine leukemias and preliminary assessment of clonality by polymerase chain reaction. *Vet Immunol Immunopathol* 69:145–164.

Walton RM, Hendrick MJ: 2001. Feline Hodgkin's-like lymphoma: 20 cases (1992–1999). *Vet Pathol* 38:504–511.

Washburn KE, Streeter RN, Lehenbauer TW, et al: 2007. Comparison of core needle biopsy and fine-needle aspiration of enlarged peripheral lymph nodes for antemortem diagnosis of enzootic bovine lymphosarcoma in cattle. *J Am Vet Med Assoc* 230:228–232.

Weiss DJ: 2001. Flow cytometric and immunophenotypic evaluation of acute lymphocytic leukemia in dog bone marrow. *J Vet Intern Med* 15:589–594.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiel J, Vardiman JW (eds). pp. 109–145.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 4

HEMOSTASIS

Mary K. Boudreaux, DVM, PhD; Elizabeth A. Spangler, DVM, PhD; and Elizabeth G. Welles, DVM, PhD

ESSENTIAL CONCEPTS

Hemostasis is an intricate, highly balanced interaction between blood vessels, platelets, and soluble factors in the formation and dissolution of blood clots. These interactions maintain blood in a fluid state under physiologic conditions. Following vascular injury or other procoagulant stimuli, the process of hemostasis promotes the rapid formation of platelet plugs and clots to minimize blood loss. Platelets plug endothelial wounds and provide a surface for the assembly of coagulation factors as well as soluble factors that promote hemostasis. Coagulation is the process that results in generation of thrombin, a multifunctional plasma enzyme that converts soluble fibrinogen into insoluble fibrin. Concurrent generation of plasmin, another plasma enzyme, causes dissolution of fibrin via fibrinolysis. Disorders of hemostasis or unbalanced hemostasis may lead to hypocoagulation (hemorrhage) or hypercoagulation (thromboembolic disorders). The processes of coagulation and fibrinolysis also are involved in tissue inflammation and repair, tumor metastasis, and reproductive processes such as ovulation and implantation.

ENDOTHELIUM

The surface of endothelial cells is a major site of procoagulant and anticoagulant interactions. Antithrombotic properties of the vascular endothelium include production of inhibitors of blood coagulation and platelet aggregation, modulation of vascular tone and permeability, and provision of a protective surface over reactive subendothelial structures, such as subendothelial collagen. Endothelial cells also can be stimulated by cytokines and other mediators to initiate a procoagulant response.

I. ANTITHROMBOTIC PROPERTIES OF ENDOTHELIAL CELLS INCLUDE THE FOLLOWING:

A. Prostacyclin (PGI_2) release. Prostacyclin is a prostaglandin that activates adenylate cyclase and increases cyclic adenosine monophosphate (cAMP) production, which results in vasodilation and platelet inhibition.

B. Nitric oxide (endothelium-derived relaxing factor) release. Nitric oxide functions similarly to prostacyclin as an inhibitor of platelet function and a potent vasodilator.

C. Thrombomodulin expression. Thrombomodulin acts as an anticoagulant by binding thrombin and inhibiting the enzyme's ability to function in the coagulation system or activate platelets. Thrombin bound to thrombomodulin activates Protein C, which in concert with protein S inhibits coagulation

www.Ebook777.com

factors V and VIII. Thus, thrombomodulin modulates thrombin activity and converts it from procoagulant to anticoagulant.

D. Tissue plasminogen activator (tPA) release. tPA converts plasminogen to plasmin in the presence of fibrin, which initiates fibrinolysis.

E. Heparan sulfate expression. Heparan sulfate accelerates antithrombin binding and inactivation of thrombin and factor X.

F. Tissue factor pathway inhibitor (TFPI) synthesis. TFPI inhibits tissue factor/activated factor VII complex.

G. EctoADPase release. EctoADPase degrades locally generated adenosine diphosphate (ADP), which limits platelet aggregation.

II. PROCOAGULANT PROPERTIES THAT FOLLOW STIMULATION OF ENDOTHELIAL CELLS INCLUDE THE FOLLOWING:

A. Tissue factor synthesis. Tissue factor in combination with factor VIIa and factor X forms the extrinsic factor X activation complex.

B. von Willebrand factor (VWF) synthesis, storage and release. VWF supports platelet adhesion to subendothelial collagen. The largest multimeric and most functional forms of VWF are synthesized and stored in Weibel-Palade bodies within vascular endothelial cells and are released in response to a variety of stimuli.

C. Plasminogen activator inhibitor type 1 (PAI-1) synthesis and release. PAI-1 inhibits fibrinolysis.

D. Damage to the endothelium results in the following:
1. Loss of protective vascular endothelial lining
2. Exposure of the subendothelium with enhanced platelet adherence and aggregation
3. Reduction of cell-membrane-associated thrombomodulin and heparan sulfates that inhibit hemostasis

PLATELETS

I. MORPHOLOGY

A. Mammalian platelets are small, anuclear, cytoplasmic fragments of megakaryocytes. They average 3 to 5 μm in diameter in most species and have fine reddish granules. Feline platelets are more variable in size and may be as large as erythrocytes. Feline platelets have the largest mean platelet volume (MPV) in health. Equine platelets stain more faintly with Romanowsky stains and may be difficult to identify on blood smears.

B. Platelets have a phospholipid bilayer membrane that contains transmembrane and peripheral glycoproteins. These glycoproteins serve as receptors for activation, adhesion, and aggregation.

C. Platelet form is maintained by a submembranous microtubule coil. Many transmembrane receptors are linked to the cytoskeleton by actin-associated proteins.

D. There are three types of membrane-bound cytoplasmic granules:
1. Alpha granules appear reddish or azurophilic in Romanowsky-stained platelets. These are the largest and most numerous granules as observed by light microscopy. They contain coagulation and growth factors and proteins involved in platelet adhesion, aggregation, and tissue repair. Examples

Free ebooks ==> www.Ebook777.com

include fibrinogen, factor V, VWF, thrombospondin, platelet factor 4, and platelet-derived growth factor (PDGF).

2. Dense granules primarily store adenine nucleotides, calcium, inorganic phosphates, and serotonin. Results of organelle proteomic studies suggest these granules also may contain proteins not previously documented, including cell signaling proteins, molecular chaperones, cytoskeletal proteins, and proteins involved in glycolysis.

3. Lysosomes, the third type of granule, contain acid-dependent hydrolases including glycosidases, proteases, and lipases.

E. Avian thrombocytes usually are oval, measure 5 to 6 µm in diameter and have a high nuclear:cytoplasm ratio, round nuclei with densely clumped chromatin, and clear, colorless cytoplasm that may contain one or more distinct magenta to purple granules. Thrombocyte-specific granules contain serotonin and thromboplastin. Analogs to some mammalian glycoproteins have been identified in avian thrombocyte cell membranes.

II. PRODUCTION

A. Platelets are produced by extension of megakaryocyte cytoplasm into vascular sinuses within bone marrow. Proplatelets fragment into individual platelets in circulation.

1. Megakaryocytes are derived from a bipotent megakaryocyte-erythroid progenitor (MEP). Although the MEP was once thought to arise from a committed common myeloid progenitor, there is recent evidence that suggests the MEP may arise directly from an uncommitted short-term hematopoietic stem cell.

2. Two morphologically distinct colonies that lead exclusively to megakaryocyte production have been identified *in vitro*. The burst-forming unit megakaryocyte (BFU-MK) is considered a primitive progenitor cell and produces complex colonies that contain several hundred megakaryocytes, which include satellite colonies. The colony-forming-unit-megakaryocyte (CFU-MK) is a more mature progenitor and divides into colonies containing 3 to 50 megakaryocytes.

3. As megakaryocytes mature, cell division ceases but DNA continues to replicate via endomitosis until a ploidy of 8N-32N (or rarely 64N or 128N) is reached.

4. Nuclear and cytoplasmic maturation are independent; initiation of cytoplasmic maturation may overlap with terminal rounds of endomitosis.

a. Stage I megakaryocytes (MK) are 15 to 50 µm in diameter with a 5-µm rim of intensely basophilic cytoplasm and oval, round, or kidney-bean shaped nuclei.

b. Stage II MK are up to 75 µm in diameter and have increased nuclear lobulation, basophilic cytoplasm, and a few azurophilic granules.

c. Stage III MK are up to 150 µm in diameter and have lobulated nuclei, variable amounts of eosinophilic cytoplasm, and numerous azurophilic granules. They are capable of platelet production.

5. Maturation time from stage I MK to platelet release is approximately 4 to 5 days (in people).

B. Platelet circulating lifespan is approximately five to nine days in most animal species.

C. The spleen normally contains up to 30% to 40% of the circulating platelet mass. Epinephrine-induced splenic contraction from excitement, fear, pain, or exercise may cause increased platelet counts. Conversely, splenic congestion or hypersplenism may sequester sufficient platelets to cause thrombocytopenia.

D. Regulation of platelet production includes the following:

1. Thrombopoietin (TPO) is a key humoral regulator of platelet production. TPO is critically important for all stages of megakaryopoiesis. TPO is also important for overall hematopoiesis and plays a role in enhancing expansion of hematopoietic stem cells.

www.Ebook777.com

a. Under steady state conditions, TPO is produced constitutively and bound by platelets and megakaryocytes via specific TPO receptors referred to as c-Mpl. Bound TPO is internalized and degraded and not available for stimulation of thrombopoiesis. TPO levels increase during episodes of thrombocytopenia, which results in enhanced thrombopoiesis.

b. TPO concentration is inversely correlated to the mass of megakaryocytes and platelets. TPO concentration is not related to platelet number.

c. Splenic sequestration or release of platelets may alter platelet number in blood, but has no effect on total platelet mass. Therefore, splenic sequestration of platelets will not affect platelet production.

2. While TPO production is constitutive under steady state conditions, severe thrombocytopenia can result in enhanced production of TPO by bone marrow stromal cells. Inflammatory conditions can result in enhanced TPO production by hepatocytes, thought to be mediated by IL-6, with resultant reactive thrombocytosis.

3. Other cytokines involved in megakaryopoiesis and platelet production include stromal cell derived factor 1 (SDF-1), chemokine receptor 4 (CXCR4), fibroblast growth factor-4 (FGF-4), IL-3, GM-CSF, stem cell factor (SCF), IL-11, IL-12, IL-1-alpha, and leukemia inhibitory factor (LIF).

E. Early avian thrombocyte precursors are derived from mononuclear precursors; however, the committed stem cell has not been identified positively in avian bone marrow. Late thrombocyte precursors are stimulated by thrombocyte colony stimulating factor. Cellular maturation progresses from thromboblast to immature thrombocyte to mature thrombocyte.

III. FUNCTION

A. Platelet shape change. Platelets exposed to various stimuli undergo several physiologic events including shape change, adhesion, aggregation, granule secretion, and expression of phosphatidylserine, which facilitate assembly of coagulation factors on their surface.

B. Platelet shape changes from discoid to spherical with formation of pseudopodia.

C. Platelet adhesion

1. Platelets adhere to exposed subendothelial collagen, VWF, fibronectin, and vitronectin. Membrane glycoproteins (GP) including GPIb-IX-V, GP-VI, and integrins $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) participate in platelet adhesion events.

2. Platelet glycoprotein Ib-IX-V (GPIb-IX-V) complex, which interacts with collagen indirectly, is critically important in initiation of platelet contact with collagen under high shear conditions. GPIb-IX-V complex mediates transient arrest of platelets from flowing blood and weak tethering of platelets onto exposed subendothelial surfaces through binding of VWF. As tethered platelets roll along exposed subendothelium they encounter collagen fibrils, which bind to GPVI. This leads to inside-out signaling and activation of integrins $\alpha2\beta1$ and $\alpha IIb\beta3$, release of ADP, and thromboxane formation, which in turn reinforce GPVI interactions. Activated $\alpha2\beta1$, transformed to a high affinity state, binds tightly to specific sequences in collagen and allows firm platelet adhesion and spreading.

D. Platelet aggregation. Activated $\alpha IIb\beta3$ is transformed to a high affinity state, and reinforces firm platelet adhesion and platelet aggregation by binding to fibrinogen.

E. Granule release

1. Aggregating platelets have rapid granule release; the rate and degree of granule release is agonist-type and agonist-concentration dependent.

2. In general, binding of various agonists activates platelet phospholipases, which result in calcium and diacylglycerol mobilization and synthesis of thromboxane A_2 (TXA_2).

a. TXA_2 induces irreversible platelet aggregation and release.

Free ebooks ==> www.Ebook777.com

b. ADP, serotonin, and coagulation factors, including fibrinogen, factor V, and factor XI, are released from granules and enhance further platelet activation and thrombus growth.
c. Platelet activation results in phosphatidylserine translocation to the outer membrane. Phosphatidylserine facilitates assembly of coagulation factor complexes, which leads to thrombin generation.

F. Platelets play an essential role in inflammation through release of vasoactive compounds (such as serotonin and platelet activating factor [PAF]), the production of cytokines, and interactions with neutrophils. They are also involved in tissue repair owing to release of potent mitogens, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF).

G. Avian thrombocyte aggregation is mediated through fibrinogen receptors that are expressed during activation in a similar manner to those of mammalian platelets. Thrombocyte aggregation is induced by thrombin, collagen, serotonin, and arachidonic acid.

IV. LABORATORY EVALUATION OF PLATELETS

A. Platelet count
1. Analysis methods
a. Automated platelet counts can be performed on platelets collected in anticoagulated whole blood. The anticoagulant of choice is EDTA. Several instruments that use different methods are available:
(1) Aperture impedance flow automated hematology instruments (Coulter Counter, Heska CBC-Diff™, Heska Hematrue™, SCIL ABC Counter, CDC Mascot Hemovet Counter, Abaxis HMII and HMV, and others). Platelets are measured by size. Coefficients of variation of approximately 5% are commonly reported (good accuracy) for most animal species except cats.
(a) Because cats have variably sized platelets, some of which are very large, there can be overlap with counting of erythrocytes. Cat platelet counts can be falsely low (pseudothrombocytopenia) because platelets are miscounted as erythrocytes.
(b) Aggregated platelets also may cause inaccurate counts because clumps may be counted as erythrocytes or leukocytes, or excluded, depending on their size. Platelet aggregation can occur in any species, but is a common problem in feline blood specimens.
(c) Vortex mixing of cat blood specimens with platelet aggregation may result in higher platelet counts, but results are too variable and unreliable to recommend this as a corrective procedure.
(d) Collection of cat blood samples in a citrate-based anticoagulant containing platelet inhibitors such as theophylline, adenosine, and dipyridamole (Diatube-H, Becton Dickinson. Oxford, UK) decreases platelet aggregation and pseudothrombocytopenia.
(e) If platelet aggregates are observed on blood smears, then platelet counts as determined by impedance counters or flow cytometric analysis are falsely decreased.
(f) Platelet counts obtained on blood by quantitative buffy coat (QBC) analysis are not affected by platelet clumping as observed with impedance counters, but all QBC analysis-derived platelet counts have only fair to good accuracy (the coefficient of variation is quite high in comparability studies, see below).
(2) Quantitative buffy coat analysis (IDEXX QBC VetAutoReader).
(a) This method is based on differential centrifugation of blood components in samples of anticoagulated whole blood by use of modified microhematocrit tubes.
(b) Blood components are stained with acridine orange, a fluorescent dye.
(c) A plastic cylindrical float with a density similar to that of platelets and leukocytes expands the buffy coat, which allows quantification of platelets and leukocytes based on their differential fluorescence. DNA primarily stains green, while RNA, lipoproteins, and granules containing glycosaminoglycan primarily stain orange to red.

www.Ebook777.com

(d) This method of platelet quantification has fair accuracy with blood specimens from dogs, horses, and cats. QBC determination of platelet mass (plateletcrit) is not influenced by platelet aggregation as with impedance counters.

(e) The QBC instrument cannot be used for ruminant blood because cellular components do not separate well by differential centrifugation.

(3) Flow cytometry (Siemens ADVIA® 120 hematology instrument, IDEXX LaserCyte®).

(a) Platelet count is determined based on light scattering characteristics.

(b) Accuracy of platelet counts is very good to excellent with most species; aggregation of platelets in feline specimens is less of a problem but still exists and blood smears must be examined for platelet clumping.

b. Manual counting by use of a hemocytometer.

(1) Whole blood is diluted and erythrocytes are lysed with an ammonium oxalate diluent solution (Unopette system®).

(2) Platelets are counted by light or phase contrast microscopy.

(3) Precision of the platelet counts is fair to poor and coefficients of variation typically range from 20% to 25%.

(4) Platelet clumping interferes with accuracy because large aggregates are excluded from the counting area (depth only 0.1 mm) and the number of individual platelets in smaller aggregates is difficult to determine.

(5) Avian thrombocyte counts can be performed by use of Natt and Herrick's diluent. Thrombocytes may be difficult to distinguish from small lymphocytes and young erythrocytes.

c. EDTA-induced platelet clumping has been reported occasionally in horses and dogs with resultant spuriously low platelet counts (pseudothrombocytopenia). Citrate anticoagulated whole blood may be used for more accurate counts in these situations.

d. Manual and automated platelet counts may be compared with estimated platelet counts on stained blood smears (see below).

(1) Cat platelet counts are unreliable due to platelet clumping, and must be verified by estimation of platelets on stained blood smears.

(2) Cavalier King Charles Spaniels often are thrombocytopenic. By use of impedance and flow cytometric counters, the degree of thrombocytopenia appears worse than it is because giant platelets, which are common in this breed, are excluded from the count.

2. Interpretation

a. Platelet counts substantially below the reference interval for a given species (typically less than 100,000/μL) indicate thrombocytopenia. Greyhounds are exceptions. In health, typically they have lower platelet counts than other breeds of dogs; therefore, a diagnosis of thrombocytopenia requires platelet counts to be considerably lower than 100,000/μL. Spontaneous petechial to ecchymotic hemorrhage usually does not occur until the platelet count is below 20,000/μL.

b. Platelet counts that exceed the reference interval (typically more than 800,000/μL) indicate thrombocytosis. Thrombocytosis associated with inflammation (reactive thrombocytosis) usually is not associated with increased risk of thrombosis. However, thrombocytosis associated with myeloproliferative disease may increase risk of thromboembolic disease.

B. Platelet evaluation from stained blood smears

1. Platelet number may be reported as decreased, within the reference interval, or increased based upon observation of platelets on stained blood smears. Decreased platelet counts reported from automated hematology instruments because of platelet aggregation in the blood sample usually are correctly estimated as normal to increased based on evaluation of stained blood smears.

2. Several techniques are used to estimate platelet numbers in blood smears:

a. Platelet estimation can be determined based on the average number of platelets/100 × oil immersion field (OIF) by light microscopy.

(1) 8 to 10 platelets/100 × OIF is greater than or equal to 100,000/μL

Free ebooks ==> www.Ebook777.com

(2) 6 to 7 platelets/100 × OIF is approximately equal to 100,000/μL
(3) <3 to 4 platelets/100 × OIF indicates significant thrombocytopenia
(4) On blood smears, each platelet/100 × OIF corresponds to approximately 20,000 platelets/μL; therefore, 10 platelets/100 × OIF equals 200,000 platelets/μL. Blood smears with platelet aggregates are usually associated with adequate platelet numbers. In cats where automated counters report low platelet counts, estimation of platelets from blood smears should always be done.

b. Less than 1 platelet/50 erythrocytes (in the monolayer of a blood smear) denotes thrombocytopenia, but presence of anemia must be considered to evaluate severity.

c. Platelet count also can be estimated on a stained blood smear by use of the following formula:

$$(\text{number of platelets/WBC observed}) \times (\text{WBC count/}\mu\text{L}) = \text{estimated platelet count/}\mu\text{L}$$

d. In healthy birds, thrombocyte counts range from 20,000 to 30,000/μL. The estimated thrombocyte count is within the reference interval if there are 10 to 15 thrombocytes/1,000 RBCs or 1 to 2 thrombocytes/OIF in the monolayer area of the smear.

3. Platelet morphology

a. Giant platelets (large platelets, shift platelets, macroplatelets, megathrombocytes) may be found in thrombocytopenia caused by excessive platelet consumption or destruction with intact bone marrow production. They may be round or elongated.

b. Platelet fragments (microplatelets or platelet microparticles) are less than 1 μm in diameter and may occur in iron-deficiency anemia (often with thrombocytosis), bone marrow aplasia, immune-mediated thrombocytopenia (associated with activation of canine platelets), or as an artifact of *in vitro* storage and aging in EDTA anticoagulant for more than 24 hours.

c. Platelets with decreased granularity, vacuoles, or less intense staining may be seen in situations where platelet activation has occurred *in vivo* (e.g., FeLV, DIC) or *in vitro* (e.g., poor specimen handling or collection).

C. Mean platelet volume (MPV)

1. MPV, measured in femtoliters (fL), is determined by impedance and optical particle counters. MPV reflects the average size of platelets in the circulating population. MPV generally is inversely proportional to platelet number.

2. Increased MPV with thrombocytopenia suggests responsive thrombopoiesis. Larger platelets (giant platelets, shift platelets) are fully functional and usually hyper-responsive. MPV greater than 12 fL indicates normal to increased megakaryocytopoiesis in bone marrow with 96% positive predictive value.

3. Decreased MPV most commonly is associated with insufficient megakaryocytes, lack of a megakaryocyte response (bone marrow failure), and early immune-mediated thrombocytopenia.

4. Increased MPV should be expected in disorders with responsive thrombopoiesis (e.g., immune-mediated thrombocytopenia). Megakaryocyte number and morphology should be evaluated in bone marrow aspirates, especially in patients with low MPV and thrombocytopenia or in patients who fail to respond to treatment.

5. Platelets sphere and swell in EDTA anticoagulated blood samples that are stored for more than four hours, cooled to room temperature (25°C), or refrigerated (4°C). Swelling may result in an artifactually high MPV. Platelets retain their native discoid shape and have minimal MPV changes when collected in citrate anticoagulant and stored at 37°C.

D. Platelet distribution width (PDW), a number without specific units, is determined by some cell counters and is a measure of platelet size variation.

E. Reticulated platelets, which contain increased RNA, have been released recently from bone marrow (less than 24 hours old). They may be quantitated by flow cytometric analysis using thiazole orange, a fluorescent dye that binds RNA. Increased reticulated platelets suggest responsive thrombopoiesis. This assay is rapid and simple if proper equipment is available and less invasive than bone marrow aspiration.

www.Ebook777.com

F. Mean platelet component concentration (MPC) is determined by modified two-angle light scatter (Siemens ADVIA® 120 hematology instrument). The MPC is linearly related to both platelet refractive index and platelet density. Only unaggregated platelets are included in the analysis. MPC also may be useful in determination of the state of activation of platelets. Two non-overlapping populations of feline platelets suggest non-activated platelets (MPC 14.8 to 18.5 g/dL) and activated platelets (MPC 20.2 to 21.6 g/dL) (data from comparative study with ADVIA®).

G. Antiplatelet antibody

1. Numerous assays (platelet immuno-radiometric assays, microscopic and flow cytometric platelet immunofluorescence assays, direct megakaryocyte immunofluorescence assay, enzyme-linked immunosorbent assays, and platelet factor 3 or PF3 assay) have been developed to identify antibodies or antigen-antibody complexes on platelets or megakaryocytes as the cause of primary or secondary immune-mediated thrombocytopenia. These assays are available in some specialized laboratories; however, most of these tests are insensitive and non-specific. For example, the PF3 assay has highly variable (28% to 80%) sensitivity. Most dogs with immune-mediated thrombocytopenia are negative upon testing; therefore, the PF3 assay cannot be recommended from a diagnostic standpoint.

2. Direct determination of platelet surface-associated immunoglobulin (PSAIg) by fluorescence labeling and flow cytometry may distinguish some cases of immune-mediated thrombocytopenia from non-immunologic thrombocytopenia. The PSAIg assay cannot differentiate primary from secondary immune-mediated thrombocytopenia.

a. Platelet glycoproteins (such as the fibrinogen receptor GP IIb-IIIa) are common targets of antibodies. Non-specific immune complexes also may be bound to platelet Fc receptors or adsorbed on platelet surfaces.

b. If platelets must be washed for this assay, artifactually high concentrations of PSAIg may be detected. Platelet activation and alpha granule release add granule membrane with potential antigens to the platelet surface.

c. PSAIg is best measured in samples less than four hours old, which limits test availability to locations where the assay is performed.

d. Samples stored for more than 24 hours have increased PSAIg; however, interpretation of test values may still be valid if based on 24-hour reference intervals, which can be established in the individual laboratory.

3. Diagnosis of primary immune-mediated thrombocytopenia is made by process of elimination.

H. Platelet function assays are available in some institutions where platelet studies are conducted. Samples must be analyzed within three hours of collection. These assays are not adapted to commercial or diagnostic laboratories.

1. Platelet aggregation is considered the gold standard for assessment of platelet function. Platelet aggregometers assess the ability of platelets to form aggregates by use of light transmission (platelet-rich-plasma) or impedance (whole blood) as monitoring methods. Platelet release can be performed simultaneously to evaluation of platelet aggregation responses (^{14}C-serotonin release, luciferin/luciferase assays). Species-specific differences must be accommodated and validated for methods of isolation of PRP as well as interpretation of responses to various agonists. These tests are useful in identification of:

a. Hereditary platelet functional defects (e.g., canine thrombopathia, Glanzmann thrombasthenia, etc.)

b. Acquired disorders of platelet function secondary to disease or drug administration (e.g., uremia or aspirin or nonsteroidal anti-inflammatory drug administration)

2. Aperture closure instruments. The Platelet Function Analyzer–100® (PFA-100®; Dade-Behring) is an example of an aperture closure instrument that can be used to document global, high-shear-dependent, platelet adhesion and aggregation. The instrument aspirates citrated whole blood under high shear through a capillary tube and a compartment containing test cartridge membranes. Test

Free ebooks ==> www.Ebook777.com

cartridge membranes have a small centrally located aperture and are coated with either ADP and collagen or collagen and epinephrine. As blood flows under high shear across the membrane and through the aperture, platelets become activated and begin to adhere and aggregate, which results in closure of the aperture within one to three minutes. The instrument measures decrease in flow rate with time until flow completely stops. The final closure time (CT) and volume of blood flow are recorded by the instrument. CT is affected by many variables including platelet count, hematocrit, medications, von Willebrand disease, intrinsic platelet disorders, and improper sample handling including errors in anticoagulant-to-blood ratios. Two different platelet agonists are available in the kit reagents: epinephrine and ADP. Epinephrine does not produce reliable results in dogs because it only potentiates aggregation and is not a true agonist. ADP, as agonist, gives reproducible results (typically 50 to 85 seconds).

I. Bleeding time

1. Bleeding time measures the length of time (minutes) required for platelets to plug a small laceration in blood vessels. This *in vivo* assay evaluates primary hemostasis or platelet status. Test sensitivity is low, especially if cuticle bleeding time is used.

2. Different bleeding time techniques have been used including cuticle bleeding time (clipping a toenail into the quick). Toenail clipping is not recommended because variation in amount of toenail removed influences bleeding time and the procedure is very painful.

3. Buccal mucosal bleeding time (BMBT) is the most reliable clinical test for *in vivo* assessment of platelet function. A spring-loaded cassette produces a precise depth and length of cut on the buccal mucosa of an extremely quiet or anesthetized animal. Anesthesia is recommended to avoid licking, head shaking, and excitement, all of which affect the length of time required for primary hemostasis to occur.

4. BMBT is prolonged in thrombocytopenia; performance of the test does not provide new diagnostic information.

5. BMBT should be reserved for use in animals with adequate platelet count but questionable platelet function.

6. BMBT may be prolonged with acquired or congenital platelet dysfunction such as VWD, acquired or congenital, and acquired platelet disorders from effects of medications or immune-mediated disorders.

7. Coagulation factor deficiencies do not prolong BMBT.

8. Vitamin C deficiency (scurvy) with resultant loss of vascular collagen integrity causes prolonged bleeding time in guinea pigs and people, but has not been reported in domestic animals.

J. Clot retraction

1. This test measures platelet function and may be performed in a clinical setting. A sample of blood (0.5 mL) is drawn directly into a plastic syringe that contains cold saline (4.5 mL) and is mixed. A portion of this blood/saline mixture (2 mL) is placed in glass tubes (test is performed in duplicate) that contain a small amount of thrombin (0.1 ml of 10 units/mL), capped, mixed, and refrigerated for 30 minutes. The tubes are then placed in a 37°C water bath, and clot retraction is graded +1 to +4 (depending on tightness of the clot and amount of clear serum surrounding the clot) at one and two hours (Figure 4.1).

2. The clot retraction test should be reserved for animals with a known adequate platelet count and questionable platelet function (as with bleeding time). The test should not be performed on animals with administration of known antiplatelet drugs (aspirin, NSAIDs). Thrombocytopenia will result in poor clot retraction.

3. This test depends on the interaction between platelet receptors, thrombin, and fibrinogen; therefore, some specific platelet functional defects will not be detected.

a. Normal clot retraction occurs in the CalDAG-GEFI platelet disorders (canine thrombopathias) because these platelets can interact with thrombin and express fibrinogen receptors.

www.Ebook777.com

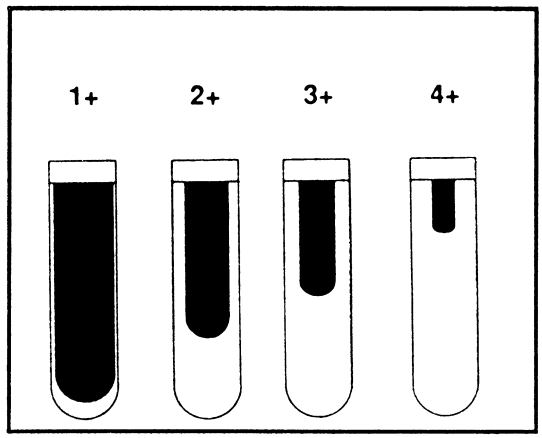


FIGURE 4.1. Clot retraction evaluation scale, +1 to +4.

b. Platelets from dogs with VWD also react normally with thrombin.
c. Abnormal clot retraction may be seen in dogs and horses with Glanzmann thrombasthenia.

K. VWF assays

1. There are three forms of VWD: Type 1, Type 2, and Type 3 (see VWD under platelet disorders for more information).

2. VWF assays are available in several laboratories. Tests are quantitative ELISA assays, which require citrated or EDTA plasma, frozen in plastic tubes after immediate separation from erythrocytes, and shipped frozen to the laboratory within two weeks of collection. Serum is not an acceptable sample; VWF is partially consumed during clot formation; thus, serum values will be artifactually low.

3. The collagen-binding activity assay (CBA) is a method for evaluation of VWF function and is useful for identification of dogs with acquired or inherited Type 2 VWD. CBA ELISA assays use collagen-coated microtiter plates to assess for VWF binding capacity. Calculations of ratios of VWF-antigen to VWF-CBA can be used to distinguish Type 2 VWD from Types 1 and 3. Dogs with Type 2 VWD have VWF antigen assay results that are discordant with their CBA results due to reduced presence of the more functional high molecular weight multimers of VWF. Ratios of VWF-antigen to VWF-CBA greater than 2 are consistent with the diagnosis of Type 2 VWD. To distinguish acquired and inherited forms of Type 2 VWD requires treatment of potential underlying causes of acquired VWD and subsequent re-evaluation of the VWF-antigen to VWF-CBA ratio.

4. Botrocetin and ristocetin assays may be performed as qualitative assays in some species but these methods are not readily available and require expertise in performance and interpretation.

5. VWD molecular assays are available for some breeds and, when available, are recommended for accurate identification of carrier states. The website vetgen.com may be consulted for current breed-specific molecular assays.

L. Flow cytometry assays

1. Flow cytometric studies can be useful for initial characterization of novel platelet function disorders in animals, particularly when the patient is not near a laboratory that tests platelet function.

2. Flow cytometry can determine if platelets lack major glycoproteins such as GPIIb and GPIIIa if species-sensitive antibodies are available.

Free ebooks ==> www.Ebook777.com

3. Flow cytometry can assess function by detection of binding of fibrinogen to activated platelets. CAP1 is an antibody that detects fibrinogen bound to activated canine platelets. CAP1 does not bind to native fibrinogen; therefore, platelet washing prior to assessment is not necessary.
4. Flow cytometric assays can be performed successfully on samples that are 24 hours old if care is taken during collection and shipping (collect samples atraumatically into CPDA-1 and do not expose to cold temperatures). Sample volume required for these assays is much less than for traditional platelet aggregation studies.

V. DISORDERS OF PLATELETS

A. Functional impairment of platelets and thrombocytopenia may cause bleeding.
   1. Bleeding usually involves body surfaces or mucous membranes.
   2. Petechial or ecchymotic hemorrhage usually is observed.

B. Thrombocytosis and enhanced platelet function may increase the risk of thrombosis.

C. Extrinsic platelet dysfunction
   1. von Willebrand disease (VWD)
      a. von Willebrand factor (VWF) is synthesized predominantly by megakaryocytes and endothelial cells.
      b. VWF is a large multimeric plasma glycoprotein needed for normal platelet adhesion to subendothelial collagen and serves as a carrier for factor VIII-coagulant (FVIII:C).
      c. VWF provides stability for FVIII:C in circulation.
      d. Small, medium, and large multimers of VWF exist; the largest multimers are most active in hemostasis.
      e. VWD may be inherited or acquired.
         (1) The specific defect involves VWF, a plasma protein.
         (2) VWF is necessary for adequate platelet function.
         (3) Platelet numbers are usually within the reference interval.
      f. VWD is relatively common in dogs (more than 50 breeds have been reported with VWD), but is rare in cats, horses, and cows. Pigs often are used as an animal model of human VWD.
      g. FVIII:C activity often is decreased because of insufficient VWF to stabilize and protect the protein from degradation by proteases. Usually more than 30% to 40% FVIII:C activity exists in affected dogs (even dogs with Type 3 or severe VWD); thus, APTT is not prolonged in canine VWD.
      h. Clinical signs of VWD may include mucosal hemorrhage (e.g., epistaxis, gastrointestinal bleeding, hematuria, and excessive bleeding from tooth eruption), prolonged bleeding from wounds, and increased cutaneous bruising (e.g., after venipuncture or clipping).
      i. Severity of clinical signs varies greatly from an absence of apparent bleeding to severe bleeding. Concurrent thrombocytopenia, disease conditions that inhibit platelet function, or drug administration that inhibits platelet function exacerbate bleeding. A plasma transfusion or cryoprecipitate is sufficient for treatment and provides VWF and FVIII:C. DDAVP, which releases preformed high molecular weight VWF multimers from Weibel-Palade bodies, may be effective in controlling hemorrhage during minor surgical procedures in dogs with Type 1 VWD.
      j. Type 1 VWD is a partial quantitative deficiency of VWF; plasma concentrations of all size multimers are less than 50%.
         (1) Clinical signs of bleeding are not observed until VWF concentrations are below 20%.
         (2) VWF that is present is structurally and functionally normal.
         (3) Greater than 90% of all cases of VWD in dogs are Type 1.
         (4) Type 1 VWD has an autosomal pattern of inheritance; males and females are equally affected.

www.Ebook777.com

(5) Severity of disease can vary greatly.

k. Type 2 VWD has qualitative abnormalities of VWF structure and function.

(1) Decreased plasma VWF concentration is associated with disproportionate loss of high molecular weight multimers.

(2) Type 2 VWD has an autosomal recessive inheritance pattern.

(3) Type 2 VWD is rare in dogs, but has been described in German Shorthaired Pointers and German Wire-haired Pointers.

l. Type 3 VWD is a severe quantitative deficiency of VWF (sometimes referred to as severe type 1 VWD).

(1) VWF concentration is usually less than 0.1% of normal.

(2) Dogs have reduced FVIII:C, but usually greater than 30% activity remains; therefore, APTT is not prolonged.

(3) Type 3 VWD has an autosomal recessive pattern of inheritance.

(4) Type 3 VWD has been reported in Chesapeake Bay Retrievers, Scottish Terriers, Shetland Sheepdogs, and Dutch Kooikers.

m. Acquired VWD has been documented in dogs and can in situations where there is excessive high shear stress (such as in severe aortic stenosis or problems where blood is jetting through narrow orifices), which results in unfolding of VWF and immediate cleavage by ADAMTS13 (a disintegrin and metalloprotease with thrombospondin repeats; ADAMTS13). ADAMTS13 is an enzyme that cleaves VWF into smaller, less functional, multimers when VWF is under high shear stress or involved in platelet aggregates (these situations expose cleavage sites that ADAMTS13 recognizes). ADAMTS13 is necessary to prevent excessive platelet adhesion or aggregation mediated by large VWF multimers.

D. Intrinsic platelet dysfunction

These are disorders due to defects in platelets themselves and may involve platelet granules, membrane glycoproteins, signal transduction proteins, proteins involved in platelet production by megakaryocytes, or signals related to procoagulant expression. Platelet transfusions are necessary to arrest bleeding and restore adequate hemostasis for most intrinsic platelet disorders.

1. Chédiak-Higashi syndrome (CHS) is an autosomal recessive genetic disorder characterized by abnormal leukocyte, melanocyte, and platelet granulation. Platelets of affected animals lack discernable dense granules and are deficient in storage pools of adenine nucleotides, serotonin, and divalent cations. Affected individuals have a diluted coat color (smoke blue Persian cats, fawn colored Hereford cattle, silver-blue Arctic foxes) and may experience prolonged bleeding at surgical incision sites and hematoma formation at venipuncture sites. CHS has been diagnosed in mink, cattle, foxes, killer whales, and mice. CHS in cattle, mice, and people has been linked to mutations in the lysosomal trafficking regulator (LYST) gene.

2. Glanzmann thrombasthenia (GT) is a deficiency of glycoprotein (GP) complex IIb-IIIa (integrin $\alpha_{IIb}\beta_3$) on platelet surfaces. Platelets are unable to bind fibrinogen.

a. Impaired or absent platelet aggregation and abnormal clot retraction are observed.

b. GT has been documented at the functional, biochemical, and molecular level in Otterhounds (previously known as thrombasthenic thrombopathia), Great Pyrenees dogs, a Quarter horse-cross, a Thoroughbred, a Peruvian Paso, and an Oldenburg.

3. CalDAG-GEFI platelet disorders, also known as canine thrombopathia, Basset Hound thrombopathia, Spitz thrombopathia, Landseer-ECT thrombopathia, and bovine thrombopathia, are inherited signal transduction platelet disorders.

a. Platelets exhibit abnormal fibrinogen receptor exposure and impaired dense granule release. These abnormalities are caused by absent or dysfunctional calcium diacylglycerol guanine nucleotide exchange factor–I (CalDAG-GEFI), a signal transduction protein important in the pathway leading to the change in conformation of GPIIb-IIIa necessary for fibrinogen binding.

Free ebooks ==> www.Ebook777.com

b. Affected animals experience mucosal bleeding and petechial and ecchymotic hemorrhages, which intensify with trauma or surgery.

c. Diagnosis of novel cases requires platelet aggregation testing or flow cytometry; clot retraction is normal in these disorders.

d. The molecular basis for these disorders has been determined in Basset Hounds, Eskimo Spitz, Landseers-ECT, and Simmental cattle.

4. A platelet dense granule defect has been described in a family of American Cocker Spaniels. Although platelet counts are within reference interval and platelet morphology appears normal, ADP concentration is decreased and an altered ATP/ADP ratio exists.

5. Cyclic hematopoiesis is an autosomal recessive disorder described in Grey Collies characterized by cyclic fluctuations in numbers of circulating neutrophils, reticulocytes, and platelets. Melanocytes are also affected in this disorder. The basis for the disease is a bone marrow stem cell defect, which results in neutropenic episodes approximately every 12 days. Mortality is high; most puppies die prior to six months of age due to fulminating infection. Platelet numbers usually are not below reference interval, but fluctuate between 300,000 and 700,000/μL. Platelet reactivity to collagen, PAF, and possibly thrombin is defective. Platelet dense granules are absent. Clot retraction and platelet adhesiveness are impaired. A mutation in the gene that encodes adaptor protein complex 3 (AP3) beta-subunit has been linked to this disorder. AP3 directs trans-Golgi export of transmembrane cargo proteins to granules.

6. Leukocyte adhesion deficiency type III (LAD-III or LAD-I variant) disorder is due to Kindlin-3 deficiency or dysfunction. Kindlin-3 has been identified as a key signal transduction protein critical for activation of beta subunit-type integrins (β1, β2, and β3) located on hematopoietic cells, including neutrophils and platelets. Kindlin-3 deficiency is characterized by lack of ability of platelets and leukocytes to activate their beta subunit-type integrins. Affected individuals bleed in a manner similar to that observed with Glanzmann thrombasthenia (GT), have persistent leukocytosis, and are susceptible to infections as observed with LAD-I. A mutation in the gene encoding Kindlin-3 has been identified in a German Shepherd dog with abnormal bleeding, persistently increased leukocyte counts, and chronic infections.

7. P2Y12 is one of two key platelet ADP receptors. P2Y12 activation does not contribute to platelet shape change, but is associated with platelet aggregation and granule release, thromboxane generation, expression of procoagulant activity, and inhibition of adenylate cyclase. A mutation in the gene encoding P2Y12 has been identified in a Greater Swiss Mountain dog. The affected dog's platelets did not bind CAP1 in response to high concentrations of ADP, but did bind CAP1 in response to convulxin and PAF in a manner similar to healthy controls. Prolonged postoperative hemorrhage was the primary clinical sign.

8. Scott syndrome is a rare bleeding disorder owing to lack of procoagulant expression on the surface of platelets. The disorder has been described in a family of German Shepherd dogs. Platelet aggregation, clot retraction, and BMBT are normal. Annexin-5 binding by ionophore activated platelets, as assessed by flow cytometry, is markedly diminished in affected dogs, which suggests lack of phosphatidylserine exposure. Bleeding in affected dogs is similar to that seen in dogs with coagulopathies due to impaired assembly of coagulation factor complexes.

9. Macrothrombocytopenia of Cavalier King Charles Spaniels (CKCS) is due to a mutation in the gene encoding beta1-tubulin. The mutation is thought to affect microtubule stability and alters megakaryocyte proplatelet formation and release. Affected dogs typically have platelet counts that range from 30,000 to 100,000/μL. However, plateletcrit determined by quantitative buffy coat (QBC) method (*I*DEXX VetAutoread™) typically is normal. Affected dogs do not have bleeding tendencies; however, because the cause of their thrombocytopenia may be misdiagnosed, they are at risk for receiving inappropriate medical (steroids and/or antibiotics) or surgical (splenectomy) treatments. Some cross-bred CKCS dogs or dogs with similar ancestry as CKCS likely carry this mutation, either in heterozygous or homozygous form, but at a much lower rate (unpublished data, MK Boudreaux).

www.Ebook777.com

E. Acquired qualitative functional disorders

1. Hyporesponsive platelets

a. Drugs

(1) Cyclooxygenase inhibitors

(a) Aspirin irreversibly acetylates cyclooxygenase within platelets and megakaryocytes and results in inhibition of thromboxane A_2 production. Because megakaryocytes are also affected, abnormal platelet function may be apparent for five days.

(b) Ibuprofen and other nonsteroidal anti-inflammatory drugs (NSAIDs) reversibly inactivate cyclooxygenase. This effect usually lasts less than six hours, depending on the half-life of the drug.

(c) Almost all cyclooxygenase-2 (COX-2) selective NSAIDs have some COX-1 inhibition activity. Therefore, COX-2 NSAIDs have a slight effect on platelet metabolism and function.

(d) These drugs, when administered at recommended dosages, do not result in clinically significant bleeding unless the patient has an underlying platelet problem such as VWD, thrombopathia, or thrombocytopenia. Elective surgery should be avoided during the period of drug treatment.

(2) β-lactam antibiotics, such as penicillins and cephalosporins, reversibly inhibit platelet function by binding agonist receptors, which impairs agonist-induced Ca^{+2} flux across the platelet membrane. These effects are not usually clinically relevant.

(3) Calcium channel blockers such as barbiturates, diltiazem, nifedipine, and verapamil prevent Ca^{+2} movement across membranes, which impairs platelet activation. Effects of calcium channel blockers vary depending upon the specific drug, dosage, and species of animal.

b. Uremia may impair platelet function and prolongs BMBT. Mechanisms are complex and in people may include:

(1) Inhibition of fibrinogen binding because of altered function of GPIIb-IIIa receptor or the presence of interfering substances that bind to the receptor.

(2) Decreased platelet reactivity because of increased cytosolic cAMP due to increased prostacyclin production and nitric oxide secretion

(3) Altered VWF–platelet interactions.

(4) Similar mechanisms are suspected to occur in animals, but have not yet been documented.

c. Disseminated intravascular coagulation (DIC) may produce increased plasma concentration of FDPs. FDPs may competitively inhibit fibrinogen binding to platelet receptors, which impairs platelet aggregation.

d. Liver disease may be associated with impaired platelet function; however, mechanisms are poorly understood.

e. Infectious and miscellaneous agents that may impair platelet function include the following:

(1) FeLV infection may cause thrombocytopenia, thrombocytosis, and/or impaired platelet function.

(2) *Ehrlichia canis* infection may cause inhibition of platelet adhesion and/or aggregation because of hyperproteinemia or the presence of anti-platelet receptor antibodies. These effects may occur in the absence of thrombocytopenia.

(3) Paraproteinemia of plasma cell myeloma may inhibit platelet adhesion and/or aggregation.

(4) Leukemia and myeloproliferative disorders may be associated with various platelet defects including altered signal transduction pathways, altered membrane glycoproteins, or abnormal platelet granules.

(5) Some snake venoms contain platelet inhibitory peptides or enzymes that degrade platelet membrane receptors and/or VWF.

2. Enhanced platelet function

a. Nephrotic syndrome may cause platelet hyperreactivity; the mechanism is likely multifactorial. Findings from one study showed platelet hyperaggregability diminished when plasma albumin concentration increased.

Free ebooks ==> www.Ebook777.com

b. In dogs, erythropoietin administration increases the number of reticulated platelets in circulation, which have increased reactivity upon exposure to agonists.

c. Infectious agents and parasites may be associated with hyperreactive platelets.

(1) FIP virus may affect platelets directly, but the increased risk of thrombosis is probably the result of endothelial cell perturbation or inflammation induced by viral infection.

(2) Heartworm disease may enhance platelet aggregation and granule release. Excreted parasite products, endothelial damage, and erythrolysis may contribute to enhanced platelet reactivity. Treatment with aspirin at recommended dosages may not effectively inhibit platelet activity in this disease.

F. Mechanisms of thrombocytopenia. The four basic mechanisms that may produce thrombocytopenia include decreased production, increased consumption, sequestration, and excessive loss. Many of the disorders that cause thrombocytopenia involve more than one of these mechanisms. The following outline categorizes diseases that cause thrombocytopenia by their major mechanism.

1. Decreased platelet production or failure of platelet production (decreased bone marrow megakaryocytes with concurrent thrombocytopenia)

a. Pure megakaryocytic hypoplasia (dogs) is a rare form of immune-mediated thrombocytopenia in which the autoantibody is directed against megakaryocytes. Bone marrow evaluation is required to make this diagnosis. Anecdotally, this disorder responds poorly to treatment. It also may be associated with immune-mediated hemolytic anemia.

b. Bone marrow panhypoplasia (pancytopenia, aplastic anemia) of any cause will result in thrombocytopenia. Bone marrow panhypoplasia begins with leukopenia (neutropenia) because neutrophils have the shortest lifespan in circulation (approximately 10-hour circulating half-life). Thrombocytopenia is apparent within several days because of longer platelet lifespan (three to 10 days, depending on species). Progressive nonregenerative anemia develops over the next few weeks (onset of anemia is variable, depending on erythrocyte lifespan; see Chapter 1). Causes of bone marrow panhypoplasia include (also see Chapters 1 and 2):

(1) Drugs, such as chemotherapeutic agents, chloramphenicol, sulfadiazine, estrogen compounds (dogs), and griseofulvin (cats) cause direct cytotoxic effects on bone marrow progenitor cells. The drug effects may be predictable, such as in cancer chemotherapy, or idiosyncratic.

(2) Chemicals such as trichloroethylene and benzene

(3) Mycotoxins and plant toxicosis, such as aflatoxin B and bracken fern ingestion, respectively

(4) Exposure to ionizing radiation

(5) FeLV or parvoviral infections may cause pancytopenia through destruction of hematopoietic cells.

c. Myelophthisis (bone marrow space is occupied by neoplastic or other non-marrow cells)

(1) Neoplastic causes include myeloproliferative or lymphoproliferative diseases, which originate within the marrow cavity, or a variety of metastatic neoplasms that infiltrate the marrow cavity.

(2) Stromal proliferation may obliterate the marrow cavity; either deposition of collagen (myelofibrosis) or osteoid (osteosclerosis).

(3) Disseminated inflammation of the marrow cavity (e.g., granulomatous osteomyelitis with disseminated histoplasmosis) also may cause myelophthisis.

d. Infectious agents

(1) Late stages of ehrlichiosis and other rickettsial diseases frequently are associated with persistent thrombocytopenia.

(2) FeLV, FIV, equine infectious anemia (EIA) virus, African swine fever virus, and bovine viral diarrhea (BVD) virus may directly infect and destroy marrow hematopoietic cells and/or may infect stromal cells, which results in decreased production of growth stimulant factors or increased production of growth suppressor factors.

www.Ebook777.com

2. Platelet consumption or destruction in excess of rate of platelet production

a. Immune-mediated thrombocytopenia

(1) In primary immune-mediated thrombocytopenia (also called autoimmune thrombocytopenia or idiopathic thrombocytopenia), autoantibodies are produced against platelet autoantigens. GP IIb-IIIa and GP Ib-IX are common antigenic targets. Megakaryocytes may be targeted along with platelets if the antibody is directed against a common membrane antigen.

(2) In secondary immune-mediated thrombocytopenia, antibody production is secondary to another underlying condition such as systemic lupus erythematosis, neoplasia, infectious disease, or drug administration. The platelet membrane nonspecifically adsorbs the offending antibody.

(3) Vaccine-induced thrombocytopenia has been associated with several different modified live vaccines.

(a) Thrombocytopenia may follow immunization against canine distemper and parvovirus in dogs, panleukopenia (parvovirus) in cats, and hog cholera in pigs.

(b) Mild thrombocytopenia occurs three to 10 days post vaccination; platelet counts are seldom below 100,000/μL. Marked thrombocytopenia may occur, but the nadir is difficult to determine because platelet counts rebound rapidly.

(c) Clinical bleeding does not occur unless an underlying platelet defect, such as VWD or thrombopathia, coexists. Elective surgery should be avoided during this time interval.

b. Drug-induced thrombocytopenia

(1) With drug-independent thrombocytopenia (primary drug-induced immune-mediated thrombocytopenia), the offending drug incites antibody formation to a normal platelet membrane antigen. Antibody production persists in the absence of the drug. Drug-independent thrombocytopenia is difficult to distinguish from primary IMT.

(2) Drug-dependent thrombocytopenia (secondary drug-induced) is observed more commonly in cats, dogs, and horses than is drug-independent thrombocytopenia.

(a) In novel or first exposure drug-dependent thrombocytopenia, the immune-mediated thrombocytopenia occurs with the first exposure to the offending drug. An example is thrombocytopenia that may follow heparin administration in horses.

(b) In previous exposure drug-dependent thrombocytopenia, the patient has a history of previous treatment with or exposure to the offending drug.

i) Thrombocytopenia is observed at least one week or more after initial drug treatment.

ii) Thrombocytopenia returns within three days of administration of the offending drug.

(c) Drug-induced non-immune mechanisms of thrombocytopenia include direct agglutination and sequestration, excessive loss, and removal via normal activation and coagulation pathways.

c. Increased activation and removal of platelets can occur with intravascular parasites (*Plasmodium* sp., *Dirofilaria immitis*); DIC; and infections with bacteria, rickettsia, and viruses.

3. Sequestration of platelets may occur with marked splenic congestion and neoplasia.

4. Excessive platelet consumption occurs with hemorrhage, trauma, rodenticide toxicosis, DIC, and neoplasia; however, thrombocytopenia is usually observed only in DIC and some cases of disseminated neoplasia.

G. Causes of thrombocytosis (platelet numbers exceed reference interval; MPV is variable). Individuals with thrombocytosis may be at increased risk of thrombosis or hemorrhage, depending on platelet function.

1. Physiologic thrombocytosis is caused by epinephrine-induced splenic contraction. In health, the spleen contains 30% to 40% of all circulating platelets. Physiologic thrombocytosis is a transient phenomenon.

Free ebooks ==> www.Ebook777.com

2. Essential thrombocythemia (ET, primary thrombophilia, hemorrhagic thrombocythemia/thrombocytosis, primary thrombocythemia, or idiopathic thrombocythemia) is a rare myeloproliferative disorder (Chapter 3).

a. Platelet count is persistently, and often markedly, increased.

b. Platelet morphologic changes include variation in size, variation in shape, and intense granulation.

c. Platelet function is abnormal in human beings, but has not been evaluated thoroughly in domestic animals.

d. Bone marrow megakaryocytes are increased, often with abnormal morphology and increased numbers of immature megakaryocytes.

e. Hemorrhage or thrombosis may occur, depending on whether platelets are hyper- or hypofunctional. Selective loss of high molecular weight VWF multimers may contribute to hemorrhage.

3. Reactive thrombocytosis (secondary thrombocytosis) is an increase in platelet count associated with conditions other than myeloproliferative disorders. Depending on the stimulus, thrombocytosis may be short term or long term. Reactive thrombocytosis is much more common than ET.

a. Inflammation and malignancy are the most common causes of reactive thrombocytosis. Cytokines associated with inflammation or secreted by tumor cells, such as IL-6, IL-3, and IL-11, stimulate megakaryocytic proliferation and maturation. IL-6 is thought to enhance thrombocytosis by stimulating TPO production by hepatocytes. The cause of thrombocytosis in iron deficiency is not known; however, enhanced thrombopoiesis may occur secondary to erythropoietin effects, possibly at the level of the MEP. Measurement of increased concentration of IL-6 has been suggested as a means to differentiate reactive thrombocytosis from ET.

b. Decreased removal or destruction of platelets by macrophages. Splenectomy is followed by thrombocytosis. Platelet counts peak about two weeks post-operatively and return to reference interval within two to three months.

H. Megakaryocytic leukemia is observed rarely (Chapter 3). Platelet counts may be decreased, within reference interval, or increased. Megakaryoblasts or micromegakaryocytes may be observed. Alterations in both platelet and megakaryocyte morphology may be present (see dysthrombocytopoiesis, below).

I. Dysthrombocytopoiesis is the disordered production of platelets. Dysthrombocytopoiesis is associated with both megakaryocytic leukemia and myelodysplastic syndromes. Myelodysplastic syndrome is characterized by cytopenia(s) in blood, hypercellular bone marrow, and dysplastic changes in one or more hematopoietic cell lines. Dysplastic changes of megakaryocytes include immaturity of cells, dispersed nuclei (multinucleated rather than multilobulated), anisokaryosis, and asynchronous nuclear to cytoplasmic maturation. These changes may be observed with FeLV infection, chemotherapeutic drug administration, cephalosporin treatment, inherited and acquired genetic mutations, and clinical disorders with markedly responsive thrombocytosis.

COAGULATION AND FIBRINOLYSIS

I. MECHANISMS OF COAGULATION

Activation of the coagulation system occurs simultaneously with platelet activation at sites of vascular injury. Coagulation is a highly regulated system, which involves formation of complexes that result in conversion of zymogens into active serine proteases. These complexes require negatively charged cell membrane phospholipid surfaces and calcium for maximum efficiency. The principle coagulation factor complexes are the intrinsic factor X activation complex (also known as intrinsic tenase complex) composed of factors IX, VIII, and X; the extrinsic factor X activation complex (known as extrinsic tenase complex) composed of factors VII, III (tissue factor), and X; and the prothrombinase complex composed of factors X, V, and II. Traditionally, the coagulation system has been divided into intrinsic, extrinsic, and

www.Ebook777.com

common pathways for ease of understanding and for laboratory testing *in vitro*. However, cross-activation does occur *in vivo* and it is known that the extrinsic factor VII-tissue factor complex, in addition to activating factor X in the common pathway, can also activate factor IX in the intrinsic pathway (Figure 4.2).

A. Serine protease coagulation factors are synthesized by the liver and occur in plasma as proenzymes or zymogens, which require activation to become functional.

1. Enzymatic factors

a. Factor XII (Hageman factor), factor XI (plasma thromboplastin antecedent), and prekallikrein (Fletcher factor) constitute most of the contact activation system.

b. Vitamin K-dependent factors include factor II (prothrombin; activated factor II is thrombin), factor VII (proconvertin), factor IX (antihemophilic factor B, Christmas factor), and factor X (Stuart factor).

(1) Inactive precursors are made functional by vitamin-K-dependent post-translational carboxylation of glutamic acid residues on the molecules.

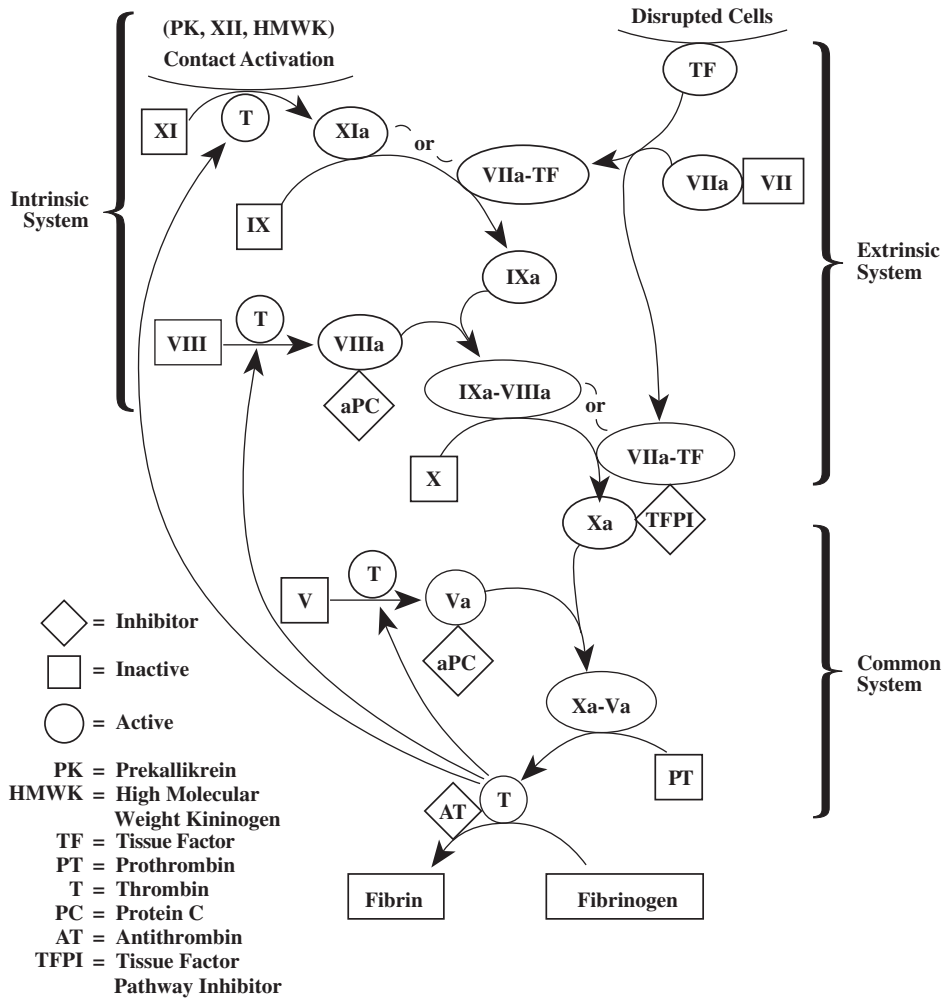


FIGURE 4.2. Scheme for activation of thrombin and fibrin formation. Notice that thrombin is a feedback activator of factors XI, VIII, and V. Activated factor VII-tissue factor complex affects the activation sequence in two locations. Other cofactors, platelet phospholipid and calcium, are not shown in this scheme.

Free ebooks ==> www.Ebook777.com

(2) Carboxylated factors II, VII, IX, and X have a negative charge and can bind to exposed phosphatidylserine on activated platelets in the presence of calcium. Calcium ions allow the electrostatic interaction to occur between negatively charged coagulation factors and negatively charged platelet surface.

(3) In cases of vitamin K antagonism (e.g., coumarin toxicosis), coagulation factors are synthesized without sufficient carboxylation and have inadequate function. These proteins are termed "proteins induced by vitamin K absence or antagonism" (PIVKA). Severity of dysfunction is proportional to degree of vitamin K deficiency (Case 25).

c. Factor XIII (fibrin stabilizing factor) is converted to an active transglutaminase by thrombin and functions to cross-link and stabilize fibrin monomers.

2. Plasma half-life of the zymogens varies from hours to a few days. Factor VII, which circulates in both zymogen and active states, has the shortest half-life (four to six hours).

3. Natural *in vivo* inhibitors of serine protease clotting factors include antithrombin (AT), whose activity is enhanced by heparin and other serpins such as heparin cofactor II.

a. The major function of AT is to inhibit thrombin activity by formation of 1:1 stoichiometric thrombin-AT complexes (TAT).

(1) Complexes are cleared by the mononuclear phagocytic system.

(2) AT activity is enhanced by heparin. The structure of AT is modified when bound with heparin, which augments AT ability to bind with thrombin.

(3) AT also inactivates other serine proteases, including activated factors IX, X, XI and XII.

b. Thrombin that escapes the site of clot formation and AT inhibition moves downstream and binds with thrombomodulin, a protein expressed on the surface of endothelial cells.

(1) Once bound to thrombomodulin, thrombin's procoagulant activity is lost.

(2) Thrombin's substrate specificity also changes from soluble fibrinogen to protein C (a vitamin-K-dependent proenzyme with anticoagulant and profibrinolytic functions, described below).

4. Activated enzymatic factors are not consumed by clotting and are present in serum.

B. Nonenzymatic protein coagulation factors are synthesized primarily by the liver and circulate in blood. Two of these proteins serve as critical cofactors within the intrinsic and extrinsic tenase and prothrombinase complexes.

1. Nonenzymatic protein factors include factor V (proaccelerin), factor VIII (antihemophilic factor A), and fibrinogen (factor I).

a. Fibrinogen and factor VIII are acute phase proteins; their concentration increases in plasma during inflammation or neoplasia.

b. The plasma half-life of these factors is hours to days.

2. Clotting consumes the nonenzymatic factors; therefore, they are absent in serum.

3. Activated factors V and VIII are degraded enzymatically by activated protein C.

a. Inactivation of factors V and VIII is accelerated in the presence of phospholipid, calcium, and protein S (a vitamin-K-dependent cofactor for protein C).

b. Inactivation of factors V and VIII results in severe impairment of the intrinsic tenase and prothrombinase complexes where these proteins serve vital roles as protein co-factors, thus explaining the anticoagulant effect of protein C.

C. Platelet phospholipid

1. Platelet phospholipid, specifically phosphatidylserine, is a cofactor of coagulation. Phosphatidylserine is expressed on activated platelet membranes and provides a negatively charged surface upon which coagulation factor activation is greatly enhanced.

2. Calcium provides a link between the negatively charged vitamin K-dependent coagulation factors and platelet membrane phosphatidylserine residues, thus positioning these enzymes and their protein cofactors for highly efficient activation of their respective substrates.

www.Ebook777.com

D. Tissue factor (factor III, tissue thromboplastin)

1. Tissue factor (factor III) is a transmembrane cell surface receptor. Tissue factor does not require proteolytic cleavage to become active. It is not exposed to plasma normally and is present in cells in the adventitia around blood vessels and in epidermal and mucosal lining cells. Tissue factor also may be expressed on the surface of monocytes/macrophages and cancer cells in certain situations and may be synthesized by platelets.

a. TF is found in subendothelial fibroblasts, pericytes, macrophages, and monocytes, but not on unperturbed endothelial cells. This contrasts with other coagulation proteins, which circulate in plasma.

b. TF synthesis by endothelial cells can be induced by cytokines, endotoxin, and other inflammatory mediators, but does not initiate coagulation without disruption of the endothelium.

c. Once exposed to blood, TF binds factor VII and activated factor VII (FVIIa) with high affinity and specificity. The TF/factor VIIa complex subsequently activates factor X and is termed extrinsic factor X or extrinsic tenase complex. TF/FVIIIa also can activate factor IX in the intrinsic tenase complex.

2. Tissue factor pathway inhibitor (TFPI) is a lipoprotein-associated protease.

a. TFPI is found predominately in platelets and endothelial cells.

b. TFPI exerts its inhibitory action in a two-step process. First, TFPI binds to activated factor X at its catalytic site. Second, this newly formed complex binds to membrane-bound FVIIa/TF complex in a calcium-dependent manner with resultant loss of FVIIa/TF catalytic activity.

E. Calcium (factor IV)

1. *In vivo* hypocalcemia does not directly result in hemorrhage. The concentration of calcium required for muscular contraction, including cardiac function, is greater than that required for blood coagulation *in vivo*.

2. Anticoagulants such as EDTA, oxalate, or citrate prevent clotting by binding calcium.

II. MECHANISMS AND DISORDERS OF THE EXTRINSIC PATHWAY (FACTORS III AND VII) OF COAGULATION (FIGURE 4.2)

A. The extrinsic pathway usually initiates coagulation *in vivo*. Coagulation is initiated when tissue factor (factor III) contacts and complexes with factor VII or activated factor VII (FVIIa). Factor VII has minimal protease activity despite the capability of autoactivation. The complex of activated factor VII and tissue factor has greatly enhanced proteolytic activity. In the presence of calcium and phospholipid (extrinsic tenase complex), this complex can activate factor IX of the intrinsic tenase complex and factor X within the prothrombinase complex.

B. Disorders of extrinsic coagulation

1. Hereditary deficiency of factor VII (dogs) is a mild disease associated with easy bruising but lacks more serious bleeding tendencies.

2. Early vitamin K inhibition or deficiency

a. Early vitamin K inhibition or deficiency initially affects factor VII because of its short half-life. Prothrombin time (PT) may be prolonged. Clinical hemorrhage may not be present.

3. Hypercoagulation and DIC caused by excessive tissue factor release (e.g., intravascular hemolysis, massive necrosis, trauma, septicemia, endotoxemia; Case 11).

III. MECHANISMS AND DISORDERS OF THE INTRINSIC PATHWAY (HMWK, PREKALLIKREIN, AND FACTORS XII, XI, IX, AND VIII) OF COAGULATION (FIGURE 4.2)

A. Contact activation involves factor XII, factor XI, prekallikrein (Fletcher factor), and cofactor high-molecular-weight kininogen (HMWK, Fitzgerald factor). These molecules are activated when

Free ebooks ==> www.Ebook777.com

plasma interacts with negatively charged substances *in vivo* (e.g., collagen, activated platelets, endotoxin) or *in vitro* (e.g., glass, kaolin, or celite).

1. The product of contact activation of the intrinsic system is activated factor XI, which subsequently activates factor IX.
2. Physiologic significance of contact activation in coagulation is controversial because isolated deficiencies of these factors usually do not result in hemorrhagic tendencies.

B. In conjunction with thrombin-activated factor VIII, activated factor IX subsequently activates factor X in the presence of calcium and phospholipid, which forms an active intrinsic tenase complex that initiates the common pathway of coagulation.

C. Blood coagulation *in vivo* probably begins after tissue injury via extrinsic tenase complex activation of factors X and IX. Sustained generation of activated factor X, however, depends on the intrinsic tenase complex, which is not susceptible to TFPI and is 50 times more efficient in activation of factor X.

1. Activated factor X (generated by the extrinsic tenase complex) activates platelets via formation of a small amount of thrombin in their vicinity.
2. Activated factor IX (generated by the intrinsic tenase complex) ultimately enhances production of thrombin on surfaces of activated platelets. Briefly, activated factor IX produces activated factor X. Activated factor X is a key component of the prothrombinase complex on the surface of platelets. The prothrombinase complex, as its name indicates, cleaves prothrombin to produce thrombin.

D. Deficiency of factors within the intrinsic pathway may or may not result in hemorrhage, and when present, will vary in severity. Coagulation-type hemorrhage is characterized by delayed deep tissue hemorrhage and hematoma formation.

E. Disorders of factors within the intrinsic pathway include:

1. Hereditary deficiency of prekallikrein (dogs and horses). Affected animals are not reported to experience clinical bleeding.
2. Hereditary factor XII deficiency or Hageman's disease (cats and dogs). Affected animals do not exhibit clinical bleeding. The abnormality usually is encountered as a serendipitous observation of prolonged activated clotting time (ACT) or activated partial thromboplastin time (APTT) in absence of bleeding.
3. Hereditary factor XI deficiency (dogs and Holstein cattle). Affected dogs and cattle have protracted bleeding after surgery but otherwise the disorder is mild.
4. Hereditary, sex-linked, factor IX deficiency or hemophilia B (cats and dogs). Affected males usually have less than 10% normal factor IX activity. Clinical signs vary with the size and activity of the animal and factor activity present. Cats with severe hemophilia B (less than 1% activity) are more likely to have a normal lifespan than a large dog with similar factor IX activity. Clinical signs tend to be non-specific, particularly in cats, and include depression, anorexia, and irritability. Spontaneous hemorrhage occurs, particularly in animals with very low factor activity. Spontaneous external hemorrhage, however, is rare; most spontaneous hemorrhages are internal and clinical signs tend to reflect the location of hemorrhage (lameness, depression, difficulty breathing, seizures, etc). Carrier females are identified by decreased activity of factor IX (usually 40% to 60%). Carriers do not experience abnormal hemorrhage.
5. Hereditary, sex-linked, factor VIII:coagulant (VIII:C) factor deficiency or hemophilia A (cats, horses, sheep, cattle, and dogs). This is the most common inherited coagulopathy of animals and people due to the high spontaneous mutation rate of the factor VIII gene. Variable severity of bleeding is similar to that observed for hemophilia B and again correlates with the severity of the factor deficiency and the size and activity of the animal. Affected males usually have less than 10% of normal factor VIII-C activity (VWF may be increased). Carrier females can be identified by decreased factor VIII:C activity (usually 40% to 60% of normal). Carrier females do not have clinical signs.

www.Ebook777.com

6. Acquired deficiency of nonenzymatic factors in disseminated intravascular coagulation syndrome (DIC) (Case 11)
   a. DIC is associated with consumption of coagulation proteins, including fibrinogen, factor V, and factor VIII. Platelets also are consumed, and excessive fibrin(ogen) degradation products (FDPs) may be generated. If clotting factor consumption exceeds their rate of synthesis, hemorrhage may result.
   b. Thrombocytopenia may be associated with petechial and/or ecchymotic hemorrhage.
   c. Thrombi may form in small vessels as part of the DIC process, which result in ischemia and end-organ necrosis and failure.
7. Acquired deficiency of vitamin-K-dependent factors (factors II, VII, IX, and X) may occur in rodenticide toxicosis (coumarin, indanedione; Case 25), fat malabsorption/maldigestion, and hepatic failure.
   a. In early rodenticide toxicosis, prolongation of the PT occurs initially because factor VII has the shortest half-life.
   b. Severity of bleeding correlates with degree of failure in carboxylation of factors.
8. Hypercoagulable states may be associated with heat stroke, viremia, and endotoxemia. These hypercoagulable states may transition into DIC depending on severity of associated tissue damage and lack of appropriate tissue perfusion.

IV. MECHANISMS AND DISORDERS OF THE COMMON PATHWAY (FACTORS X, V, II, AND FIBRINOGEN) OF COAGULATION (FIGURE 4.2)

A. Factor X, when activated by either the intrinsic or extrinsic system, binds to activated factor V, calcium, and phospholipid to form the prothrombinase complex. Factor V is present within plasma but is also concentrated within platelet α-granules.

B. The prothrombinase complex cleaves two peptide bonds in membrane-bound prothrombin, which results in formation of prothrombin fragment 1+2 and prethrombin 2. Prethrombin 2 is then cleaved to form thrombin. Thrombin dissociates from the platelet surface and converts soluble fibrinogen (factor I) to monomeric fibrin with release of fibrinopeptides A and B. Activated factor XIII (fibrin stabilizing factor) catalyzes polymerization and cross-linking of fibrin monomers to form insoluble fibrin.

C. Thrombin is a positive feedback accelerator of coagulation. It activates factors XIII, XI, VIII, V, and platelets. Thrombomodulin-bound thrombin acts as an anticoagulant by activation of protein C.

D. Disorders of the common system (factors X, V, II, and fibrinogen)
1. Disorders that only affect the common pathway are rare. Disorders that result in prolongation of both PT and APTT are usually "multiple hit" disorders such as liver disease, vitamin K antagonism, or DIC, in which factors in multiple pathways are decreased nearly simultaneously.
2. Hereditary deficiency of factor X has been described in dogs and cats.
3. Hereditary deficiency of fibrinogen has been described in goats and dogs. Fibrinogen may be absent, decreased, or abnormal. Affected animals have mild to severe bleeding.
4. Some snake venoms possess enzymes that can activate thrombin, factor V, and/or factor X.

E. Disorders in anticoagulation
1. Antithrombin deficiency. Acquired deficiency is described in dogs (protein-losing nephropathy, protein-losing enteropathy, and sepsis) and horses (colic syndrome). DIC and liver disease also may cause AT deficiency in animals. Affected animals are predisposed to thrombosis because of poorly regulated thrombin activity.
2. Protein C deficiency. Acquired deficiency of protein C has been described in horses with colic syndrome. Hereditary deficiency of protein C also may occur in horses. DIC-induced consumption of protein C has been described in dogs. Animals with hereditary or acquired deficiency of protein

Free ebooks ==> www.Ebook777.com

C are predisposed to thrombosis because of poorly regulated activity of factors V and VIII, as well as impaired control of fibrinolysis.

V. MECHANISMS AND DISORDERS OF VITAMIN-K-DEPENDENT FACTORS

A. Vitamin K antagonism, which results in inhibition of functional vitamin K-dependent factors, can occur with rodenticide toxicity. The length of time required to treat with vitamin K1 varies with the rodenticide class. First generation compounds generally require treatment for seven to 10 days; second generation compounds may require treatment for four to six weeks.

B. Vitamin K deficiency can occur rarely with malabsorption/maldigestion, including exocrine pancreatic insufficiency. Because vitamin K is continually recycled, vitamin K deficiency rarely occurs due to the time it takes to deplete vitamin K stores (approximately six months). Most animals are diagnosed and treated before vitamin K deficiency occurs.

C. Inherited vitamin-K-dependent multifactor coagulopathies have been diagnosed in Devon Rex cats, Rambouillet sheep, and a Labrador Retriever. Functional vitamin-K-dependent coagulation proteins are decreased due to mutations in the gene encoding gamma-glutamyl carboxylase. Devon Rex cats respond to vitamin K1 treatment (they synthesize carboxylase protein that has impaired function but is responsive to vitamin K1). Rambouillet sheep do not respond to vitamin K1 treatment (their mutation is a premature stop codon and they do not synthesize the carboxylase enzyme). The mutation in Labrador retrievers has not yet been described. Inherited multifactor coagulopathy should be suspected in animals that repeatedly present with vitamin-K-responsive coagulopathies in the absence of exposure to rodenticides.

VI. MECHANISMS AND DISORDERS OF FIBRINOLYSIS (FIGURE 4.3)

Fibrinolysis is initiated almost simultaneously with coagulation. It maintains blood in a fluid state within the vasculature and is responsible for patency of many tubular structures. Fibrinolysis, like coagulation, is a complex interaction and balance of inactive proenzymes, active enzymes, activators, inhibitors, and inactivators.

A. Plasminogen and plasmin

1. Plasminogen is the precursor of plasmin and is present in plasma. Plasminogen is activated to plasmin when it is cleaved into heavy (A) and light (B) chains. Activators include kallikrein, tissue plasminogen activator (tPA), and urokinase plasminogen activator (uPA).

a. Lysine binding effector sites of the A chain mediate plasmin's interaction with fibrin and its plasma inhibitor, α_2-antiplasmin (A2P).

b. The B chain contains the active enzymatic site that is homologous to other serine proteases, such as thrombin.

c. The physiologic substrate of plasmin is fibrin; however, plasmin will nonspecifically inactivate other proteins including prekallikrein, HMWK, factor VIII, factor V, and fibrinogen.

2. Plasminogen activation to plasmin is usually fibrin specific.

a. Activation of fibrinolysis occurs within developing clots where plasminogen activators and plasminogen bind to fibrin.

b. After initial proteolysis of fibrin, additional binding sites are uncovered that enhance further binding of plasminogen and its activators.

3. The activity of plasmin is inhibited by its plasma serine protease inhibitor, α2AP.

a. Inhibition is achieved by formation of stable complexes between plasmin and α2AP (PAP), which are cleared by the mononuclear phagocytic system.

www.Ebook777.com

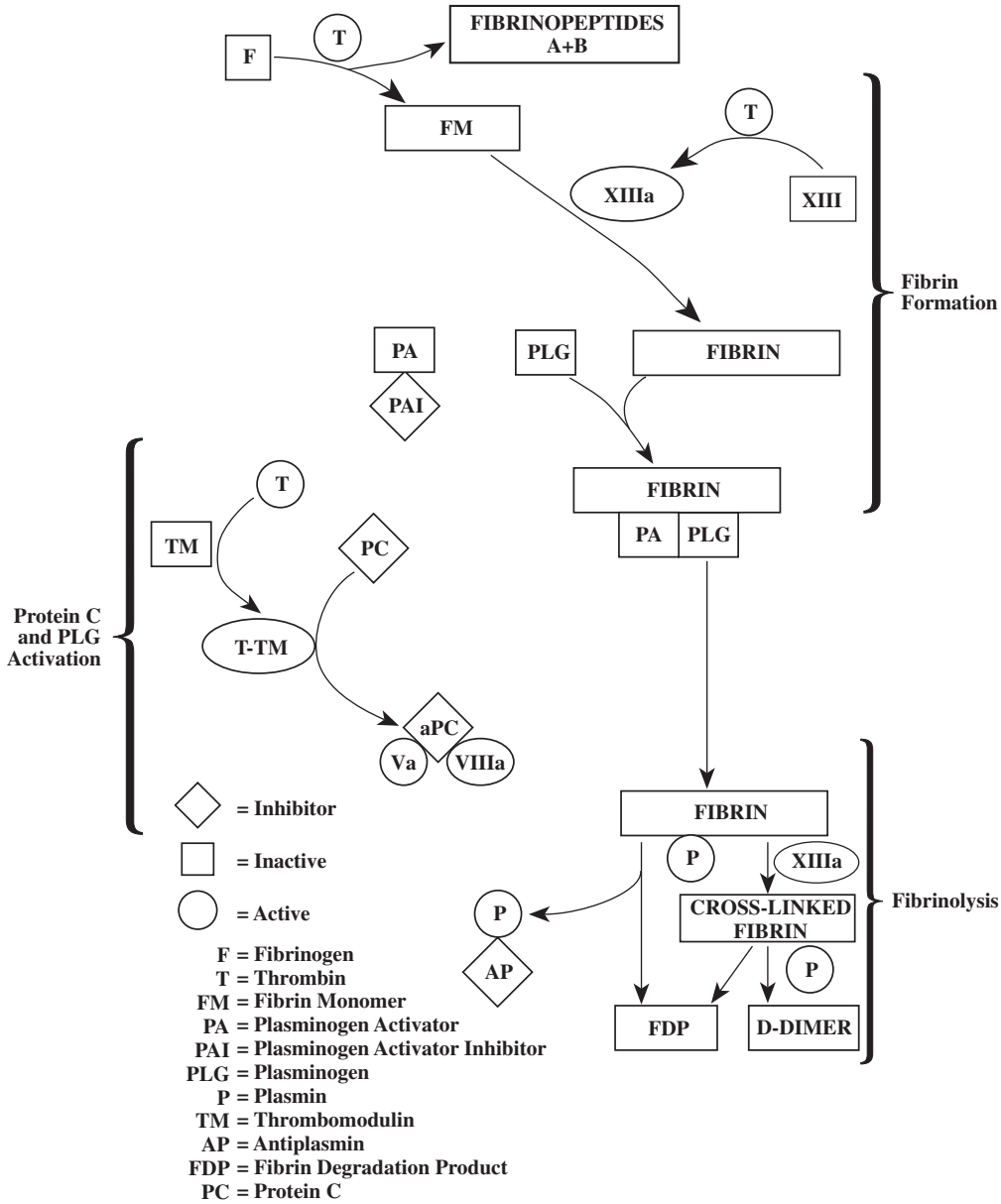


FIGURE 4.3. Scheme for fibrin formation and fibrinolysis. During formation, plasminogen activator (PA) is balanced by plasminogen activator inhibitor (PAI). Fibrinolysis proceeds with activation of plasminogen to plasmin when the proportion of PA exceeds PAI. Activated protein C (aPC) inactivates coagulation factors Va and VIIIa.

b. Fibrin-bound plasmin is site-protected from inactivation by plasma α_2-antiplasmin (α2AP); however, only partial protection is achieved because α2AP is incorporated into cross-linked fibrin by activated factor XIII (fibrin stabilizing factor).

c. Additional inhibitors of plasmin include α_2-macroglobin, α_1-antitrypsin, AT, and C1-esterase inhibitor.

Free ebooks ==> www.Ebook777.com

4. Thrombin activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase that is activated by thrombin. Activation by thrombin is greatly enhanced by binding of thrombin to thrombomodulin. Activated TAFI (TAFIa) inhibits fibrinolysis by cleaving exposed lysine binding sites on fibrin for plasminogen and tPA. TAFIa also prevents conversion of Glu to Lys-plasminogen. In disease states where thrombin is not propagated (such as hemophilia), TAFIa is not generated and fibrinolysis is enhanced.
5. Plasmin is capable of hydrolyzing fibrin and fibrinogen, with resultant formation of fibrin(ogen) degradation products (FDPs) or fragments.
   a. FDPs are cleared from circulation by macrophages (especially Kupffer cells of the liver).
   b. The terminal degradation product produced by plasmin-mediated hydrolysis of cross-linked fibrin is D-D/E fragment, which consists of two covalently bonded D-domains (D-dimer) that are bound noncovalently to fragment E.

B. Activators of plasminogen are derived from endothelium, platelets, and other cells. These activators are in plasma and efficiently activate fibrin-bound plasminogen.
1. Intrinsic activation of plasminogen
   a. Activated factor XII can directly activate plasminogen.
   b. Activated factor XII also indirectly activates plasminogen.
      (1) Activated factor XII subsequently activates prekallikrein to kallikrein.
      (2) Kallikrein activates urokinase plasminogen activator (uPA) which, in turn, activates plasminogen to plasmin.
2. Extrinsic activation of plasminogen
   a. uPA and tPA are found in many cells and have high specific activity in conversion of plasminogen to plasmin.
   b. Fibrin-bound tPA activates fibrin-bound plasminogen to plasmin. Plasmin digestion of fibrin generates new C-terminal lysine residues that serve as binding sites for more plasminogen and tPA; thus, further fibrinolysis is enhanced.
   c. uPA is primarily responsible for activation of plasminogen on cell surfaces and likely plays an important role in extravascular fibrinolysis and enhancement of cell adhesion and migration.

C. Plasminogen activator inhibitors (PAI) are derived from endothelium, platelets, and other cells.
1. PAI-1 inhibits tPA by formation of 1:1 reversible complexes that prevent plasminogen activation.
2. The balance between activators and inactivators shifts toward activators and fibrinolysis when:
   a. Inhibitors form complexes with other plasma proteins.
   b. Thrombin is released downstream and activates protein C. Protein C also enhances fibrinolysis by inhibition of thrombin generation, which in turn inhibits production of TAFI.

D. Specific disorders of fibrinolysis in domestic animals are poorly understood compared with similar human disorders. The important concept is that the components of fibrinolysis are balanced to maintain homeostasis. Imbalance of this process occurs in disease and may predispose the patient to hypercoagulable or hypocoagulable states.
1. Possible imbalances that favor hypercoagulation:
   a. Decreased plasminogen concentration (e.g., horses with severe ischemic bowel disease)
   b. Lack of plasminogen activators
   c. Lack of protein C or protein S
   d. Factor V mutations (factor V Leiden) result in resistance of factor V to inactivation by protein C
   e. Excess plasminogen activator inhibitor (PAI)
2. Possible imbalances that favor hypocoagulation:
   a. Lack of α_2-antiplasmin and other plasmin inhibitors may occur in fulminating DIC. Plasmin hydrolysis of factor V, factor VIII, and fibrinogen contribute to a hypocoagulable state.
   b. Systemic uncontrolled plasminogen activation.

www.Ebook777.com

VI. AVIAN COAGULATION AND FIBRINOLYSIS

Less is known regarding avian coagulation and fibrinolysis relative to mammals. Most studies have used human deficient plasma to determine specific avian factor activities.

A. Coagulation. Avian plasma contains most of the coagulation factors found in mammals except factor XII, prekallikrein, and HMWK. Factor XI activity also is markedly reduced. The extrinsic pathway is considered the initiator of coagulation in birds, as is the case in mammals.

B. Chicken brain tissue thromboplastin is necessary for reproducible avian PT test results.

C. AT activity has been detected in avian plasma. It behaves as an acute-phase reactant.

D. Fibrinolysis. Antiplasmin and uPA have been detected in avian plasma.

VII. LABORATORY EVALUATION OF COAGULATION AND FIBRINOLYSIS

The laboratory tests described here are effective in analysis of hypocoagulation disorders (bleeding syndromes). The differential diagnosis of important bleeding disorders by use of laboratory tests is shown in Table 4.1. Hypercoagulable states in animals that precede clinical DIC or increase the risk of thrombosis are poorly defined by most routine laboratory coagulation screening tests. Thromboelastography (TEG) is an alternative method for assessment of blood coagulation that is useful for detection of both hypocoagulable and hypercoagulable states (section VII, J).

A. Activated clotting time (ACT)

1. This test measures the time (seconds) required for fibrin clot formation in fresh whole blood after exposure to a contact activator (celite, diatomaceous earth, and/or glass tube). Platelets provide the phospholipid for assembly of coagulation complexes. The ACT evaluates the intrinsic and common pathways of coagulation.

a. The ACT is prolonged if the activity of a given factor is less than 10% of normal. Mild factor deficiencies (10% to 30% of normal activity) do not affect the ACT but will prolong the APTT. Thus, the ACT is a less sensitive test than APTT in evaluation of the intrinsic and common pathways.

b. The ACT test is easily performed in a clinical setting and provides reproducible results provided that careful attention is given to technical requirements.

(1) Two ml of whole blood, obtained from a clean venipuncture, are added to 37°C pre-warmed commercially available ACT tubes that contain diatomaceous earth (a potentiator of contact activation).

(2) Tubes are maintained at 37°C in a heat block and clot formation is checked at regular intervals by gently tilting the tube. The endpoint of the ACT test is visible clot formation.

(3) Failure to warm the tube may give a falsely prolonged ACT value because all intrinsic and common system factors are enzymes except for factors V and VIII and fibrinogen. Lower temperatures retard enzymatic activity.

(4) Reference intervals should be established for each laboratory based on ACT test values obtained from repeated testing of healthy animals with normal coagulation. Published reference intervals are inadequate for interpretation of ACT test results in a given laboratory.

c. The ACT test is easier to perform and is more sensitive than Lee-White or capillary tube clotting times because ACT tubes are commercially prepared and standardized.

2. Prolonged ACT indicates significant deficiency of coagulation factor(s) within the intrinsic (prekallikrein; HMWK; factors XII, XI, IX, VIII) or common (factors X, V, II; fibrinogen) pathways. Severe factor deficiency (less than 10% normal activity) is necessary to prolong the ACT.

3. Marked thrombocytopenia (less than 10,000/μL) may cause prolonged ACT because platelets provide the phospholipid that is required for assembly of coagulation complexes.

Free ebooks ==> www.Ebook777.com

TABLE 4.1.
PATTERNS FOR HEMOSTASIS DISORDERS.[a]

Disorder	Plt ct	BMBT	APTT (or ACT)[b]	PT	TT	FDP D-D
Disseminated intravascular coagulation (acute, uncompensated)	dec	inc	inc	inc	inc	inc
Thrombocytopenia	dec	inc	N	N	N	N
Platelet function defect	N	inc	N	N	N	N
von Willebrand disease	N	inc	N	N	N	N
Severe liver disease/insufficiency	N	N	inc	inc	N	N
Acquired vitamin K deficiency or antagonism						
Hereditary factor X deficiency						
Hereditary deficiencies of factors VIII, IX, X, or XII	N	N	inc	N	N	N
Factor VII deficiency	N	N	N	inc	N	N
Congenital fibrinogen deficiency	N	N/inc	inc	inc	inc	N

[a]Pattern is variable depending on the relative severity of the process.
[b]The ACT will be prolonged if 10% or less of intrinsic or common factor activity remains. The APTT is more sensitive and is prolonged if 30% or less of intrinsic or common factor activity remains.
Plt ct = platelet count
BMBT = buccal mucosal bleeding time
APTT = activated partial thromboplastin time
ACT = activated clotting time
PT = prothrombin time
TT = thrombin time
FDP = fibrin(ogen) degradation products
D-D = D-dimers
N = normal (within the reference interval)
inc = increased or prolonged
dec = decreased

4. The presence of coagulation inhibitors (e.g., excessive FDPs) or anticoagulants (e.g., heparin, citrate) also prolong the ACT.

5. The ACT tube also can be used for subjective assessment of clot quality. After holding the tube at 37°C, the clot should be firm with clear serum appearing within one to two hours. A soft, friable clot that liquefies in 30 to 60 minutes indicates excessive fibrinolysis or hypofibrinogenemia.

B. Citrated plasma clotting tests

1. Sample management

a. Blood should be collected via clean venipuncture with minimal venous stasis. Difficult venipuncture may promote tissue fluid contamination and hemolysis, both of which rapidly activate clotting, which may consume coagulation factors and result in falsely prolonged coagulation test times.

b. Catheters that have been used for administration of heparin or flushed with heparinized saline solutions are not recommended for sample collection. If no other site for blood collection is available, they should be used with precaution and significant blood must be withdrawn (blood

www.Ebook777.com

either can be discarded or subsequently re-administered to the patient) prior to obtainment of a sample for coagulation testing.

c. Excitement of the animal at the time of blood collection should be minimized. Excitement has been associated with increased factor VIII:C concentrations.

d. Blood is collected via clean venipuncture with a plastic syringe. Blood is immediately placed in commercial citrate tubes at precisely 9 parts fresh whole blood/1 part 3.8% trisodium citrate anticoagulant. Blood and anticoagulant should be mixed thoroughly. Failure to thoroughly mix blood and citrate anticoagulant or an incorrect blood-to-anticoagulant ratio yields unreliable test results. Drawing blood directly into syringes containing the appropriate amount of citrate can greatly facilitate collection of non-activated blood samples.

e. Plasma should be separated from cells by centrifugation within 30 minutes of sample collection.

(1) If hemostasis analyses are to be done by a distant laboratory, plasma should be frozen rapidly and kept frozen for transport to the laboratory. Slow freezing or thawing and refreezing promotes ice crystal formation and precipitation of coagulation factors (cryoprecipitation). Factor VIII:C is particularly vulnerable to these effects and APTT test results will be inconsistent.

(2) Plasma held under refrigeration at 4°C is generally stable for 48 hours.

(3) Activity of factors VII and VIII is lost quickly at room temperature, although species variation has been observed. Factor VII and VIII activity appears to be more stable in equine citrated plasma.

2. Methods

a. Reference values for citrated plasma clotting tests may change when new lots of reagents are used, even if the reagents are obtained from the same vendor.

b. Because different instruments and sources of reagents cause variations in hemostasis test results, each laboratory performing these tests should establish and provide appropriate species-specific reference intervals. Human reference intervals are inappropriate for interpretation of veterinary test results.

c. When in doubt about appropriate reference values, the patient's test results can be compared to those of a healthy animal of the same species that are determined at the same time.

d. Laboratory instruments for routine coagulation screening tests use either mechanical or optical systems to detect initial fibrin clot formation. Manual test methods are available, but are seldom used because test results are less reproducible.

(1) Optical detection of clotting is based on the increased turbidity of recalcified, citrated plasma as fibrin forms. Values from coagulation screening tests from many animal species are significantly shorter than those of humans. Therefore, optical-based instrument assays designed for human specimens must be modified to detect the earlier clot formation that occurs in animal specimens. If the assays are not modified, the clot may form in some animal specimens before the instrument can detect it and the instrument will report a "failure" of clot formation.

(2) Some veterinary hemostasis laboratories use a mechanical fibrin clot detection method (Fibrometer®) in which a hooked electrode is moved up and down through the sample next to a stationary electrode. The moving electrode is electrically activated when it is at the highest point outside the sample and inactivated when it is moving through the sample. When a fibrin strand forms in the sample, the hook pulls the fibrin strand up out of the sample mixture. The electrode becomes electrically activated above the mixture by the fibrin strand that completes a circuit between moving and stationary electrodes. The completed circuit turns off the cam and timer device and the time of the reaction is recorded.

(3) Several point-of-care instruments are available (SCA 2000 Veterinary Coagulation analyzer, Synbiotics, San Diego, CA; Idexx Coag Dx™ Analyzer, Idexx Laboratories Inc.,

Free ebooks ==> www.Ebook777.com

Westbrook, ME; Vetscan™ VSpro, Abaxis™, Union City, CA). The SCA 2000 measures ACT, PT, and APTT in non-anticoagulated whole blood (centrifugation and harvest of plasma is not required). PT and APTT tests also are available in citrate anticoagulated whole blood versions. Similarly, the Idexx Coag Dx™ has cartridges to measure PT and APTT by use of non-anticoagulated or citrated whole blood. The Vetscan™ VSpro uses a single cartridge to measure PT and APTT in citrated whole blood. Reference intervals must be established for these instruments and their reagents.

3. Activated partial thromboplastin time (APTT)

a. APTT measures time (seconds) required for fibrin clot formation in citrated plasma after addition of a contact activator of the intrinsic system, phospholipid that substitutes for platelet-derived phospholipid, and calcium.

b. Prolonged APTT indicates a coagulation factor deficiency in the intrinsic (factors XII, XI, IX, and VIII) or common (factors X, V, II, and fibrinogen) pathways.

(1) Prolonged APTT is expected in animals affected with hemophilia A or B (deficiency in factor VIII or IX), hereditary factor XII deficiency, hereditary prekallikrein deficiency, DIC, vitamin K antagonism or deficiency, and liver failure.

(2) Factor activity must be less than 30% of normal to prolong the APTT.

(a) Hemophiliac carriers (heterozygotes) do not bleed and are not detected by the APTT because they have 40% to 60% of normal factor VIII or IX activity.

(b) Animals with VWD often have decreased factor VIII:C, but activity usually does not decrease below 40% of normal. These individuals may have petechial hemorrhages but the APTT will be within reference interval (Case 28).

(c) Mild or early rodenticide toxicosis causes prolongation of PT before prolongation of APTT because factor VII has the shortest half-life of the vitamin-K-dependent factors.

(3) Thrombocytopenia does not affect APTT because the test reagents provide phospholipid. In contrast, platelets provide phospholipid for the ACT test and for clotting *in vivo*.

c. Heparin, administered for therapeutic anticoagulation, will prolong APTT.

d. Fibrinogen and factor VIII concentrations may increase in inflammatory disease with subsequent decrease in APTT, often shorter than the reference interval.

e. APTT test may be used to determine the activity of specific coagulation factors if known factor-deficient plasmas are available (see below).

f. APTT test, as typically performed, is not affected by prekallikrein deficiency. The activator commonly used in the APTT test is ellagic acid. Ellagic acid directly activates factor XII, bypassing the augmentation role of prekallikrein. Use of particulate activators, rather than ellagic acid, will result in a prolonged APTT in individuals with hereditary prekallikrein deficiency.

g. Some variation is allowed in incubation time of citrated plasma samples with test reagents prior to addition of calcium. Enough time (typically three minutes) is allowed to equilibrate the system to 37°C, but the equilibration time is not extensive enough to cause heat-induced degradation of reagents (typically five minutes). Exact incubation times prior to recalcification may be necessary to obtain reproducible APTT test results on bovine samples.

4. Prothrombin time (PT)

a. PT measures time (seconds) required for fibrin clot formation to occur in citrated plasma after addition of tissue thromboplastin (factor III) and calcium.

(1) Reagents that contain rabbit brain or synthetic tissue thromboplastin are preferred for determination of PT in mammals.

(2) Chicken brain tissue thromboplastin must be used for reliable determination of PT in birds.

b. Prolonged PT indicates coagulation factor deficiency in extrinsic (factor VII) or common (factors X, V, II and fibrinogen) pathways of coagulation.

(1) Prolonged PT is expected in hereditary factor VII deficiency, DIC, vitamin K antagonism or deficiency, and liver failure.

www.Ebook777.com

(2) Factor activity must be less than 30% of normal to prolong PT.
(3) In mild or early stages of rodenticide toxicosis, deficiency of vitamin K-dependent factor VII precedes other factor deficiencies because of its shorter half-life (approximately four to six hours). In these cases, PT may be prolonged while APTT and ACT are within reference interval. Therefore, PT is the preferred test for assessment of effectiveness of vitamin K replacement therapy.
(4) Protein C (half-life approximately eight to 10 hours), a vitamin-K-dependent antithrombotic protein, may be deficient concurrently with factor VII.
(5) Thrombocytopenia does not affect PT because test reagents include phospholipid for assembly of coagulation complexes.

5. Thrombin time (TT)
   a. TT measures time (seconds) required for fibrin clot formation to occur in citrated plasma after addition of calcium and thrombin.
   b. TT largely depends upon functional fibrinogen concentration. Hypofibrinogenemia (less than 100 mg/dL) prolongs TT. Hypofibrinogenemia may include excessive consumption of fibrinogen (DIC), hereditary deficiency of fibrinogen, or presence of dysfunctional fibrinogen (dysfibrinogenemia).
   c. Infrequently, inhibitors may be present that prolong TT.
      (1) Inhibitors may promote inactivation of thrombin (e.g., heparin) or compete with patient fibrinogen for thrombin-binding (e.g., dysproteinemias).
      (2) Sources of heparin may be from patient blood (prior administration of heparin), collection of blood specimen from an intravenous catheter that is periodically flushed with heparinized saline solution, or collection of blood specimen in the wrong anticoagulant (heparin instead of sodium citrate).
   d. Vitamin K deficiency or antagonism does not affect TT because exogenous thrombin is added for performance of the test.
6. Specific factor analysis
   a. With use of commercial immunoabsorbed plasma specimens that are deficient in a given clotting factor, the APTT test can detect specific factor deficiencies. Commercial and patient plasmas are mixed in a 1:1 ratio. If the commercial plasma and patient plasma are deficient in the same clotting factor, the APTT will be prolonged. If the commercial plasma provides the clotting factor that is deficient in the patient, the APTT will be within reference interval (normal) because more than 30% of the missing factor activity is restored.
   b. Specific factor analyses are available in specialized veterinary or comparative hemostasis laboratories. Specific factor analysis is useful to identify the most commonly inherited disorders of hemostasis in animals, which include hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency).
7. Russell viper venom test (Stypven time) bypasses the extrinsic and intrinsic pathways of coagulation and directly activates the common pathway. A prolonged Russell viper venom time implies deficiency in one or more factors (factors X, V, II or fibrinogen) within the common pathway.

C. Fibrinogen concentration (Chapter 6).
1. The most sensitive assay for detection of hypo- or dysfibrinogenemia is TT determination. TT is inversely proportional to functional fibrinogen concentration (see above discussion).
2. Heat precipitation is a relatively crude technique to measure fibrinogen concentration. It may verify the presence of normal, decreased, or increased fibrinogen concentration, but the assay is unable to distinguish normal fibrinogen from dysfunctional fibrinogen.
3. An estimate of fibrinogen concentration also can be made by calculation of the difference between plasma and serum protein concentrations.

Free ebooks ==> www.Ebook777.com

D. Fibrin(ogen) degradation products (FDPs) are produced through plasmin-mediated degradation of both fibrinogen and fibrin.

1. Methods of measurement

a. FDPs are measured in citrated plasma by use of a latex agglutination assay. Particles coated with antibody to human fibrinogen fragments agglutinate when FDPs are available for binding. Degree of agglutination provides a semi-quantitative assessment of plasma FDP concentration. The latex agglutination assay detects FDPs across species lines.

b. Because FDPs include degradation products of both fibrinogen and fibrin, they cannot be used to distinguish between primary and secondary fibrinolysis.

2. Increased FDPs

a. Increased FDP production occurs in DIC as a result of diffuse, microvascular thrombosis.

(1) In acute uncompensated DIC, FDPs are more than 20 µg/mL.

(2) In compensated DIC, FDPs may range from more than 5 to less than 20 µg/mL.

b. FDP production also may increase after severe internal bleeding or localized thrombosis (such as jugular vein thrombosis in horses).

c. FDPs may increase in patients with severe liver disease due to lack of clearance.

E. D-dimer is produced through plasmin-mediated degradation of cross-linked fibrin; therefore, D-dimer is a specific indicator of secondary fibrinolysis.

1. Methods of measurement

a. D-dimer concentration is measured in citrated plasma by use of immunologic methods that are semi-quantitative (latex agglutination) or quantitative (immunoturbidimetric). These assays use a monoclonal antibody that recognizes the D-dimer epitope.

b. Some assays for human D-dimer can be used for measurement of D-dimer concentration in citrated plasma specimens from dogs, cats, and horses. Successful application of a particular assay depends on antibody cross-reactivity between species.

2. In human patients, D-dimer is most commonly applied as a test to exclude the presence of pulmonary thromboembolism or venous thrombosis. In dogs, high D-dimer concentration has been shown to be a sensitive indicator of thromboembolic disease, including DIC.

3. D-dimer concentration may be increased in a variety of disease states that result in internal hemorrhage and thrombosis, and is not always an indicator of pathologic thromboembolism.

F. AT interacts with heparin to irreversibly inactivate thrombin and other serine proteases. AT activity is measured on automated chemistry instruments by use of a chromogenic substrate.

1. AT is a small protein synthesized by hepatocytes. AT activity may be low as a result of decreased production due to decreased liver function, consumption in hypercoagulative disorders such as DIC, or loss in protein-losing enteropathy or nephropathy.

a. In healthy individuals, AT activity typically ranges from 85% to 125% of the activity found in plasma control samples that are assayed concurrently. Control samples consist of species-specific, pooled plasma from healthy animals or human control plasma samples.

b. Animals with reduced AT activity (below 80% of the control values) are considered to be at increased risk of thrombosis.

c. Animals with AT activity of below 70% of the control value may be unresponsive to heparin treatment, and AT replacement therapy is required.

G. Thrombin-antithrombin complexes (TATs) form rapidly when thrombin is produced. Increased TAT concentration may reflect systemic activation of coagulation. TAT can be measured by ELISA or RIA assays in dogs, horses, pigs, and sheep with immunologic reagents developed for people.

H. Protein C is a vitamin K-dependent protein with anticoagulant and profibrinolytic activities.

1. This protein can be quantitated by chromogenic and Laurell rocket techniques.

www.Ebook777.com

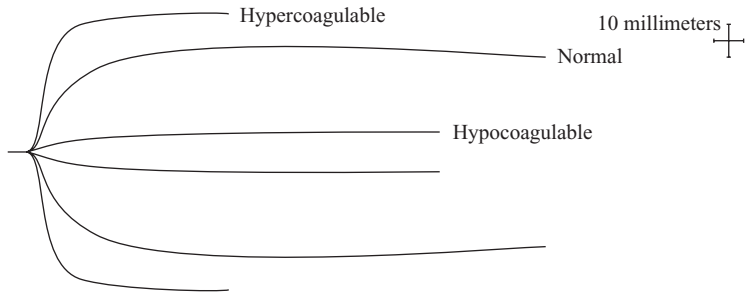


FIGURE 4.4. Comparison of TEG tracings from three dogs with varying coagulation status (hypercoagulable, normal, hypocoagulable).

2. Protein C concentration decreases in hypercoagulative disorders (e.g., septicemia in horses and dogs) and in vitamin K antagonism.

3. Hereditary deficiency of protein C is an autosomal dominant disorder; heterozygosity is associated with thrombosis in human patients. Patients with homozygous protein C deficiency develop fatal neonatal purpura fulminans (fulminant thrombosis).

I. Assays for fibrinolytic factors

1. Commercial reagent kits are available for human plasma, but fail to function with animal plasminogens. Modifications of the assay are required, including acidification and neutralization of plasma and use of urokinase as activator rather than streptokinase (the activator included in human reagent kits). Plasminogen activity has been measured in horses, cattle, dogs, and cats.

2. Assays for α_2-antiplasmin, tPA, and PAI have not been evaluated for clinical use in veterinary medicine.

J. Thromboelastography (TEG) measures viscoelastic changes that occur in blood during polymerization of fibrin. Because this method evaluates clot formation in whole blood, it provides an assessment of the influence of cellular elements of blood as well as plasma coagulation factors. TEG tracings can be used to recognize both hyper- and hypocoagulable states, and may guide decisions regarding the need for blood transfusion or other blood products (Figure 4.4).

1. Methods

a. TEG can be performed as a patient-side test with fresh whole blood, but in veterinary medicine TEG is more commonly performed with whole blood anticoagulated with sodium citrate. Anticoagulated blood samples are held for a standard period of time (approximately 30 minutes) prior to addition of calcium chloride to initiate coagulation. Activators of coagulation (kaolin or tissue factor) also may be added to the sample immediately before recalcification.

b. A computerized thromboelastograph such as the TEG 5000® (Haemoscope Corp., Niles, IL) is used for analysis. Citrated whole blood is added to a disposable cup that contains calcium chloride. A disposable pin connected to a torsion wire is lowered into the cup. The cup oscillates, and initially the blood moves freely around the pin. As the blood sample clots, the motion of the cup is impeded. This generates torque, which is detected by the torsion wire and transmitted to the computer where it is converted into a graphical representation.

c. Parameters that are measured from TEG tracings include time to initial fibrin formation (reaction time; R), time from initial clot formation until a predetermined clot strength is reached (clotting time; K), rate of clot formation (angle; α), and maximum clot strength (MA) (Figure 4.5). R and K are affected primarily by coagulation factor activity. The angle depends on coagulation factor activity, fibrinogen concentration, and platelets. MA is determined primarily by platelet number and function, with continued contributions from factors related to fibrin formation.

Free ebooks ==> www.Ebook777.com

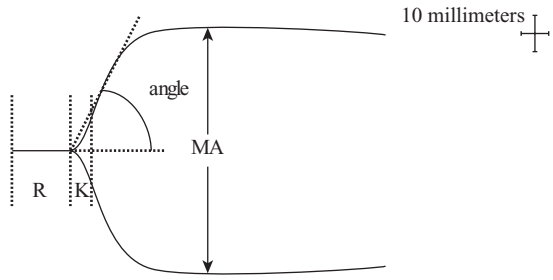


FIGURE 4.5. TEG tracing. Solid line(s) show the tracing produced by the instrument as formation of a fibrin clot is detected. Vertical lines indicate regions of the tracing that define R and K. R is the time when lines of the tracing diverge by 1 mm, which indicates initial fibrin formation. K is the time from R until the lines diverge by 20 mm and is a measure of the rate of clot formation. The angle between the baseline and a line drawn tangential to the TEG curve beginning at R is also an indication of the rate of clot formation. MA is the maximum divergence of the two lines, and provides a measure of maximum clot strength.

REFERENCES

Avgeris S, Lothrop CD, McDonald TP: 1990. Plasma von Willebrand factor concentration and thyroid function in dogs. *J Am Vet Med Assoc* 196:921–924.

Ayers JR, Leipold HW, Padgett GA: 1988. Lesions in Brangus cattle with Chediak-Higashi syndrome. *Vet Pathol* 25:432–436.

Bajaj MS, Birktoft JJ, Steer SA, et al: 2001. Structure and biology of tissue factor pathway inhibitor. *Thromb Haemostasis* 86:959–972.

Bateman SW, Mathews KA, Abrams-Ogg ACG: 1998. Disseminated intravascular coagulation in dogs: Review of the literature. *J Vet Emerg Crit Care* 8:29–45.

Benson KF, Li FQ, Person RE, et al: 2003. Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. *Nature Genetics* 35:90–96.

Berndt MC, Shen Y, Dopheide SM, et al: 2001. The vascular biology of the glycoprotein Ib-IX-V complex. *Thromb Haemostasis* 86:178–188.

Boisver AM, Swenson CL, Haines CJ: 2001. Serum and plasma latex agglutination tests for detection of fibrin(ogen) degradation products in clinically ill dogs. *Vet Clin Pathol* 30133–136.

Boudreaux MK, Dillon AR, Ravis WR, et al: 1991. The effects of treatment with aspirin and aspirin/dipyridamole in heartworm-negative, heartworm-infected, and embolized heartworm-infected dogs. *Am J Vet Res* 52:1992–1999.

Boudreaux MK, Dillon AR, Sartin EA, et al: 1991. The effects of treatment with ticlopidine in heartworm-negative, heartworm-infected, and embolized heartworm-infected dogs. *Am J Vet Res* 52:2000–2006.

Boudreaux MK, Crager C, Dillon AR, et al: 1994. Identification of an intrinsic platelet function defect in Spitz dogs. *J Vet Intern Med* 8:93–98.

Boudreaux MK, Kvam K, Dillon AR, et al: 1996. Type I Glanzmann's thrombasthenia in a Great Pyrenees dog. *Vet Pathol* 33:503–511.

Boudreaux MK, Catalfamo JL: 2001. The molecular basis for Glanzmann's thrombasthenia in Otterhounds. *Am J Vet Res* 62:1797–1804.

www.Ebook777.com

Boudreaux MK, Panangala VS, Bourne C: 1996. A platelet activation-specific monoclonal antibody that recognizes a receptor-induced binding site on canine fibrinogen. *Vet Pathol* 33:419–427.

Boudreaux MK, Lipscomb DL: 2001. Clinical, biochemical, and molecular aspects of Glanzmann's Thrombasthenia in humans and dogs. *Vet Pathol* 38:249–260.

Boudreaux MK, Catalfamo JL, Klok M: 2007. Calcium Diacylglycerol Guanine Nucleotide Exchange Factor I gene mutations associated with loss of function in canine platelets. *Translational Research* 150:81–92.

Boudreaux MK, Schmutz S, French PS: 2007. Calcium diacylglycerol guanine nucleotide exchange factor I (CalDAG-GEFI) gene mutations in a thrombopathic Simmental calf. *Vet Pathol* 44:932–935.

Boudreaux MK: 2008. Characteristics, diagnosis, and treatment of inherited platelet disorders in mammals. *J Am Vet Med Assoc* 233:1251–1259.

Boudreaux MK, Wardrop KJ, Kiklevich V, et al: 2010. A mutation in the canine kindlin-3 gene associated with increased bleeding risk and susceptibility to infections. *Thromb Haemostasis* 103:475–477.

Boudreaux MK: 2010. Platelet structure. *In:* Wardrop KJ, Weiss D (eds): Schalm's Veterinary Hematology, 6th ed. Wiley-Blackwell, Ames, pp 561–568.

Bounous DI, Stedman NL: 2000. Normal avian hematology chicken and turkey. *In:* Feldman BF, Zinkl JG, Jain NC (eds): Schalm's Veterinary Hematology, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 1147–1154.

Boutet P, Heath F, Archer J, et al: 2009. Comparison of quantitative immunoturbidimetric and semiquantitative latex-agglutination assays for D-dimer measurement in canine plasma. *Vet Clin Pathol* 38:78–82.

Brooks MB, Dodds JW, Raymond SL: 1992. Epidemiologic features of von Willebrand's disease in Doberman Pinschers, Scottish Terriers, and Shetland Sheepdogs: 260 cases (1984–1988). *J Am Vet Med Assoc* 200:1123–1127.

Brooks M: 1999. A review of canine inherited bleeding disorders: Biochemical and molecular strategies for disease characterization and carrier detection. *J Heredity* 90:112–118.

Brooks M: 2000. Coagulopathies and thrombosis. *In:* Ettinger SJ, Feldman EC (eds): Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat, 5th ed. W.B. Saunders Co., Philadelphia, pp. 1829–1841.

Brooks MB, Catalfamo JL, Brown HA, et al: 2002. A hereditary bleeding disorder of dogs caused by a lack of platelet procoagulant activity. *Blood* 99:2434–2441.

Brooks MB, Catalfamo JL, Friese P, et al: 2007. Scott syndrome dogs have impaired coated-platelet formation and calcein-release but normal mitochondrial depolarization. *J Thromb Haemostasis* 5:1972–1974.

Bruchim T, Aroch I, Saragusty J, et al: 2008. Disseminated intravascular coagulation. *Compend Cont Educ Pract Vet* 30:E1–E15.

Caldin M, Furlanello T, Lubas G: 2000. Validation of an immunoturbidimetric D-dimer assay in canine citrated plasma. *Vet Clin Pathol* 29:51–54.

Callan MB, Bennett JS, Phillips DK, et al: 1995. Inherited platelet delta-storage pool disease in dogs causing severe bleeding: An animal model for a specific ADP deficiency. *Thromb Haemostasis* 74:949–953.

Free ebooks ==> www.Ebook777.com

Callan MB, Giger U: 2001. Assessment of a point-of-care instrument for identification of primary hemostatic disorders in dogs. *Am J Vet Res* 62:652–658.

Catalfamo JL, Raymond SL, White JG, et al: 1986. Defective platelet-fibrinogen interaction in hereditary canine thrombopathia. *Blood* 67:1568–1577.

Christopherson PW, Insalaco TA, van Santen VL, et al: 2006. Characterization of the cDNA encoding αIIb and β3 in normal horses and two horses with Glanzmann thrombasthenia. *Vet Pathol* 43:78–82.

Christopherson PW, van Santen VL, Livesey L, et al: 2007. A 10-base-pair deletion in the gene encoding platelet glycoprotein IIb associated with Glanzmann Thrombasthenia in a horse. *J Vet Intern Med* 21:196–198.

Cowan SM, Bartges JW, Gompf RE, et al: 2004. Giant platelet disorder in the Cavalier King Charles Spaniel. *Exp Hematol* 32:344–350.

Davis B, Toivio-Kinnucan M, Schuller S, et al: 2008. Mutation in δ1-tubulin correlates with the macrothrombocytopenia of Cavalier King Charles Spaniels. *J Vet Intern Med* 22:540–545.

Debili N, Coulombel L, Croisille L, et al: 1996. Characterization of a bipotent erythro-megakaryocytic progenitor in human bone marrow. *Blood* 88:1284–1296.

DelGiudice LA, White GA: 2009. The role of tissue factor and tissue factor pathway inhibitor in health and disease states. *J Vet Emerg Crit Care* 19:23–29.

DiGiacomo RF, Hammond WP, Kunz LL, et al: 1983. Clinical and pathologic features of cyclic hematopoiesis in grey collie dogs. *Am J Pathol* 111:224–233.

Dodds WJ: 1997. Hemostasis. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): Clinical Biochemistry of Domestic Animals, 5th ed. Academic Press, San Diego, pp. 241–283.

Donahue SM, Otto CM: 2005. Thromboelastography: A tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. *J Vet Emerg Crit Care* 15:9–16.

Dunn JK, Heath MF, Jefferies AR, et al: 1999. Diagnostic and hematologic features of probable essential thrombocythemia in two dogs. *Vet Clin Pathol* 28:131–138.

Epstein KL, Brainard BM, Lopes MA, et al: 2009. Thromboelastography in 26 healthy horses with and without activation by recombinant human tissue factor. *J Vet Emerg Crit Care* 19:96–101.

Estrin MA, Wehausen CE, Lessen CR, et al: 2006. Disseminated intravascular coagulation in cats. *J Vet Intern Med* 20:1334–1339.

Fox JEB: 2001. Cytoskeletal proteins and platelet signaling. *Thromb Haemostasis* 86:198–213.

Frojmovic MM, Wong T, Searcy GP: 1996. Platelets from bleeding Simmental cattle have a long delay in both ADP-activated expression of GPIIb-IIIa receptors and fibrinogen-dependent platelet aggregation. *Thromb Haemostasis* 76:1047–1052.

Gentry PA, Cheryk LA, Shanks RD, et al: 1997. An inherited platelet function defect in a Simmental crossbred herd. *Can J Vet Res* 61:128–133.

Griffen A, Callan MB, Shofer FS, et al: 2003. Evaluation of a canine D-dimer point-of-care test kit for use in samples obtained from dogs with disseminated intravascular coagulation, thromboembolic disease, and hemorrhage. *Am J Vet Res* 64:1562–1569.

Hickford FH, Barr SC, Erb HN: 2001. Effect of carprofen on hemostatic variables in dogs. *Am J Vet Res* 62:1642–1646.

Jergens AE, Turrentine MA, Kraus KH, et al: 1987. Buccal mucosa bleeding times of healthy dogs and of dogs in various pathologic state, including thrombocytopenia, uremia, and von Willebrand's disease. *Am J Vet Res* 48:1337–1342.

www.Ebook777.com

Jin RC, Voetsch B, Loscalzo J: 2005. Endogenous mechanisms of inhibition of platelet function. *Microcirculation* 12:247–258.

Johnson JS, Laegreid WS, Basaraba RJ, et al: 2006. Truncated gamma-glutamyl carboxylase in Rambouillet sheep. *Vet Pathol* 43:430–437.

Johnstone IB, Lotz F: 1979. An inherited platelet function defect in basset hounds. *Can Vet J* 20:211–215.

Kaser A, Brandacher G, Steurer W, et al: 2001. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. *Blood* 98:2720–2725.

Konigsberg W, Kirchhofer D, Riederer MA, et al: 2001. The TF:VIIa complex: Clinical significance, structure-function relationships and its role in signaling and metastasis. *Thromb Haemostasis* 86:757–771.

Koplitz SL, Scott MA, Cohn LA: 2001. Effects of platelet clumping on platelet concentrations measured by use of impedance or buffy coat analysis in dogs. *J Am Vet Med Assoc* 219:1552–1556.

Kramer JW, Davis WC, Prieur DJ: 1977. The Chediak-Higashi syndrome of cats. *Lab Invest* 36:554–562.

Kristensen AT: 2000. Section VII, Hemostasis: Platelets-clinical platelet disorders. *In:* Feldman BF, Zinkl JG, Jain NC (eds): Schalm's Veterinary Hematology, 5th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 467–515.

Kunieda T, Nakagiri M, Takami M, et al: 1999. Cloning of bovine LYST gene and identification of a missense mutation associated with Chediak-Higashi syndrome of cattle. *Mammalian Genome* 10:1146–1149.

Lanevschi A, Kramer JW, Greene SA, et al: 1996. Evaluation of chromogenic substrate assays for fibrinolytic analytes in dogs. *Am J Vet Res* 57:1124–1130.

Leclere M, Lavoie U-P, Dunn M, et al: 2009. Evaluation of a modified thromboelastography assay initiated with recombinant human tissue factor in clinically healthy horses. *Vet Clin Pathol* 38:462–466.

Lipscomb DL, Bourne C, Boudreaux MK: 2000. Two genetic defects in αIIb are associated with Type I Glanzmann's thrombasthenia in a Great Pyrenees dog: A 14-base insertion in Exon 13 and a splicing defect of Intron 13. *Vet Pathol* 37:581–588.

Livesey L, Christopherson P, Hammond A, et al: 2005. Platelet dysfunction (Glanzmann's Thrombasthenia) in horses. *J Vet Intern Med* 19:917–919.

Lothrop CD, Candler RV, Pratt HL, et al: 1991. Characterization of platelet function in cyclic hematopoietic dogs. *Exp Hematol* 19:916–922.

Macieira S, Rivard GE, Champagne J, et al: 2007. Glanzmann thrombasthenia in an Oldenburg filly. *Vet Clin Pathol* 36:204–208.

Mason DJ, Abrams-Ogg A, Allen D, et al: 2002. Vitamin K-dependent coagulopathy in a black Labrador retriever. *J Vet Intern Med* 16:485–488.

Morrissey JH: Tissue factor: 2001. An enzyme cofactor and a true receptor. *Thromb Haemostasis* 86:66–74.

Nagasawa T, Hasegawa Y, Shimizu S, et al: 1998. Serum thrombopoietin level is mainly regulated by megakaryocyte mass rather than platelet mass in human subjects. *Br J Haematol* 101:242–244.

Nelson OL, Andreasen C: 2003. Utility of plasma D-dimer to identify thromboembolic disease in dogs. *J Vet Intern Med* 17:830–834.

Free ebooks ==> www.Ebook777.com

Norman EJ, Barron RCJ, Nash AS, et al: 2001. Evaluation of a citrate-based anticoagulant with platelet inhibitory activity for feline blood cell counts. *Vet Clin Pathol* 30:124–132.

Norman EJ, Barron RCJ, Nash AS, et al: 2001. Prevalence of low automated platelet counts in cats: Comparison with prevalence of thrombocytopenia based on blood smear estimation. *Vet Clin Pathol* 30:137–140.

Padgett GA, Leader RW, Gorham JR, et al: 1964. The familial occurrence of the Chediak-Higashi syndrome in mink and cattle. *Genetics* 49:505–512.

Papasouliotis K, Cue S, Graham M, et al: 1999. Analysis of feline, canine and equine hemograms using the QBC VetAutoread. *Vet Clin Pathol* 28:109–115.

Pedersen HD, Haggstrom J, Olsen LH, et al: 2002. Idiopathic asymptomatic thrombocytopenia in Cavalier King Charles Spaniels is an autosomal recessive trait. *J Vet Intern Med* 16:169–173.

Rijken DC, Lijnen HR: 2008. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemostasis* 7:4–13.

Sabino EP, Erb HN, Catalfamo JL: 2006. Development of a collagen-binding activity assay as a screening test for type II von Willebrand disease in dogs. *Am J Vet Res* 67:242–249.

Searcy GP, Frojmovic MM, McNicol A, et al: 1994. Platelets from bleeding Simmental cattle mobilize calcium, phosphorylate myosin light chain and bind normal numbers of fibrinogen molecules but have abnormal cytoskeletal assembly and aggregation in response to ADP. *Thromb Haemostasis* 71:240–246.

Sjaastad OV, Blom AK, Stormorken H, et al: 1990. Adenine nucleotides, serotonin, and aggregation properties of platelets of blue foxes (*Alopex lagopus*) with the Chediak-Higashi syndrome. *Am J Med Genetics* 35:373–378.

Smith SA: 2009. The cell-based model of coagulation. *J Vet Emerg Crit Care* 19:3–10.

Soute BA, Ulrich MM, Watson AD, et al: 1992. Congenital deficiency of all vitamin K-dependent blood coagulation factors due to a defective vitamin K-dependent carboxylase in Devon Rex cats. *Thromb Haemostasis* 68:521–525.

Steficek BA, Thomas JS, McConnell MF, et al: 1993. A primary platelet disorder of consanguineous Simmental cattle. *Thromb Res* 72:145–153.

Stokol T, Brooks MB, Erb HN, et al: 2000. D-dimer concentrations in healthy dogs and dogs with disseminated intravascular coagulation. *Am J Vet Res* 61:393–398.

Stokol T: 2003. Plasma D-dimer for the diagnosis of thromboembolic disorders in dogs. *Vet Clin N Am Small Anim Pract* 33:1419–1435.

Stokol T, Erb H, De Wilde L, et al: 2005. Evaluation of latex agglutination kits for detection of fibrin(ogen) degradation products and D-dimer in healthy horses and horses with severe colic. *Vet Clin Pathol* 34:375–382.

Tarnow I, Kristensen AT, Olsen LH, et al: 2005. Dogs with heart diseases causing turbulent high-velocity blood flow have changes in platelet function and von Willebrand factor multimer distribution. *J Vet Intern Med* 19:515–522.

Tasker S, Cripps PJ, Mackin AJ: 1999. Estimation of platelet counts on feline blood smears. *Vet Clin Pathol* 28:42–45.

Thomas JS: 1996. von Willebrand's disease in the dog and cat. *Vet Clin N Am Small Anim Pract* 26:1089–1110.

www.Ebook777.com

Topper MJ, Prasse KW: 1996. Use of enzyme-linked immunosorbent assay to measure thrombin-antithrombin III complexes in horses with colic. *Am J Vet Res* 57:456–462.

Tornquist SJ, Crawford TB: 1997. Suppression of megakaryocyte colony growth by plasma from foals infected with equine infectious anemia virus. *Blood* 90:2357–2363.

Tvedten H, Lilliehook I, Hillstrom A, et al: 2008. Plateletcrit is superior to platelet count for assessing platelet status in Cavalier King Charles Spaniels. *Vet Clin Pathol* 37:266–271.

Umemura T, Katsuta O, Goryo M, et al: 1983. Pathological findings in young Japanese black cattle affected with Chediak-Higashi syndrome. *Jap J Vet Sci* 45:241–246.

Vitrat N, Cohen-Solal K, Pique C, et al: 1998. Endomitosis of human megakaryocytes are due to abortive mitosis. *Blood* 91:3711–3723.

Wagg CR, Boysen SR, Bedard C: 2009. Thromboelastography in dogs admitted to an intensive care unit. *Vet Clin Pathol* 38:453–461.

Weiss DJ, Monreal L, Angles AM, et al: 1996. Evaluation of thrombin-antithrombin complexes and fibrin fragment D in carbohydrate-induced acute laminitis. *Res Vet Sci* 61:157–159.

Welles EG, Prasse KW, Duncan A: 1990. Chromogenic assay for equine plasminogen. *Am J Vet Res* 51:1080–1085.

Welles EG, Williams MA, Tyler JW, et al: 1993. Hemostasis in cows with endotoxin-induced mastitis. *Am J Vet Res* 54:1230–1234.

Welles EG, Boudreaux MK, Crager CS, et al: 1994. Platelet function and antithrombin, plasminogen, and fibrinolytic activities in cats with heart disease. *Am J Vet Res* 55:619–627.

Wiinberg B, Jensen AL, Rojkjaer R, et al: 2005. Validation of human recombinant tissue factor-activated thromboelastography on citrated whole blood from clinically healthy dogs. *Vet Clin Pathol* 34:389–393.

Wiinberg B, Jensen AL, Johansson PI, et al: 2008. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. *J Vet Intern Med* 22:357–365.

Wilkerson MJ, Shuman W, Swist S, et al: 2001. Platelet size, platelet surface-associated IgG, and reticulated platelets in dogs with immune-mediated thrombocytopenia. *Vet Clin Pathol* 30:141–149.

Wilkerson MJ, Shuman W: 2001. Alterations in normal canine platelets during storage in EDTA anticoagulated blood. *Vet Clin Pathol* 30:107–113.

Zelmanovic D, Hetherington EJ: 1998. Automated analysis of feline platelets in whole blood, including platelet count, mean platelet volume, and activation state. *Vet Clin Pathol* 27:2–9.

Free ebooks ==> www.Ebook777.com

CHAPTER 5

WATER, ELECTROLYTES, AND ACID BASE

Jeanne Wright George, DVM, PhD, and Shanon M. Zabolotzky, DVM

TOTAL BODY WATER AND OSMOLALITY

I. TOTAL BODY WATER (TBW)

A. TBW is approximately 60% of body weight in healthy, non-obese adult animals.

B. TBW is divided among multiple sub-volumes or spaces:
- **1.** Intracellular fluid (ICF)
- **2.** Extracellular fluid (ECF), which can be further subdivided:
  - **a.** Blood. The term "volemia" describes changes in blood volume (e.g., hypovolemia is decreased blood volume).
  - **b.** Intercellular fluid
  - **c.** Transcellular fluid, including peritoneal, pericardial, and pleural fluids. These areas are called the "third space."
  - **d.** Gastrointestinal tract. The quantity of fluid within the gastrointestinal tract can be quite large in ruminants.

C. TBW volume (hydration status) is primarily controlled by water intake (thirst) and renal output. Water loss via sweating, salivation, or panting may have minor effects.
- **1.** These control mechanisms respond to the "effective circulating volume." This is the portion of ECF that effectively perfuses tissues and stimulates volume receptors.
- **2.** The effective circulating volume is influenced not only by blood volume, but also by arterial blood pressure, arterial resistance, and delivery of blood to volume receptors.

D. Decreased total body water (dehydration) (Cases 3, 6, 8, 9, 14, 18, 22, 23)
- **1.** Dehydration is manifested as decreased body weight (e.g., 10% dehydration is a 10% decrease in body weight).
- **2.** Dehydration is best evaluated by accurate measurement of loss of body weight; this is rarely attained in clinical practice.
- **3.** Dehydration is most often inferred by clinical evidence of hypovolemia. Loss of skin elasticity, dryness of mucous membranes, retraction of eyes, and signs of shock are evidence of dehydration.
- **4.** Certain laboratory abnormalities, especially increased hematocrit, serum or plasma total proteins, albumin urea, and creatinine, along with high urine specific gravity, can help confirm the clinical finding of dehydration.
  - **a.** Many other conditions alter these values (e.g., renal disease, anemia); therefore, they should be used only in conjunction with physical examination.

www.Ebook777.com

b. Once baseline values are established for these analyses, day-to-day changes are fairly sensitive markers of changes in hydration status.

E. Increased total body water (Case 19)

1. Increased body weight is the best measure of increased body water, but it is difficult to evaluate in clinical practice.

2. Increased total body water accumulates in extracellular fluid or third spaces (e.g., edema, ascites, hydrothorax). Clinical examination is used to identify sites of fluid accumulation.

3. Hypovolemia and increased TBW may occur at the same time when water accumulates in third spaces or the gastrointestinal tract (especially in ruminants). Clinical evaluation of the patient is the best way to recognize this situation.

II. EXTRACELLULAR OSMOLALITY

A. Definitions: Osmolality is the number of solute particles/unit weight of solution. Osmolarity is the number of solute particles/unit volume of solution. For ECF, osmolality or osmolarity are about equal.

B. ECF osmolality is maintained around 300 milliosmoles per kilogram (mOsm/kg) (isomolality) in health. Changes in ECF osmolality cause shifts between the ECF and intracellular fluid (ICF), as water moves passively to the compartment with the higher osmolality.

C. Electrolytes and small molecules (e.g., glucose, urea) are the major contributors to osmolality. Large molecules (e.g., proteins) contribute little to osmolality.

D. Tonicity is the effective osmolality of a solution—the concentration of solutes that can cause shifts of H_2O across a semipermeable membrane. Only solutes that do not cross a semipermeable membrane (i.e., effective osmoles) contribute to the tonicity of a solution. Because urea freely passes between the ECF and ICF, it is an ineffective osmole that does not contribute to tonicity.

E. Serum osmolality

1. Serum osmolality is used as a measure of ECF osmolality, but it cannot be used as a measure of total body water.

2. Direct measurement, based on freezing point depression or vapor pressure, can be performed on an osmometer. Results are expressed as milliosmoles/kilogram (mOsm/kg).

3. Serum osmolality can be estimated based on the most numerous osmoles: electrolytes, glucose, and urea. Two formulas correlate well with measured osmolality.

a. For samples with normal glucose and blood urea nitrogen (BUN) concentrations:

$$\text{mOsm/kg} = 2[\text{Na}^+ + \text{K}^+(\text{mmol/L})].$$

b. For samples with either increased glucose or BUN concentration:

$$\text{mOsm/kg} = 1.86[\text{Na}^+ + \text{K}^+(\text{mmol/L})] + [\text{glucose (mg/dl)} \div 18] + [\text{BUN (mg/dl)} \div 2.8].$$

4. Ideally, the formula for calculation of osmolality should be validated for electrolyte measurement techniques used by a given laboratory.

F. Osmolal or osmolar gap

1. The numerical difference between measured osmolality and estimated osmolality is the osmolal gap (also called the osmolar gap).

2. In health, the size of the osmolal gap depends on both the method used to measure osmolality and electrolyte concentrations and the mathematical formula used to estimate osmolality. For most calculations, the osmol gap ranges from −5 to 15 mOsm/L.

3. An increased osmolal gap is an indication of the presence of unmeasured, nonpolar, low-molecular-weight substances (e.g., ethylene glycol, propylene glycol). Estimation of serum osmolality alone does not reveal this abnormality.

Free ebooks ==> www.Ebook777.com

G. Hyperosmolality

1. All animals that are hypernatremic also are hyperosmolal (Cases 29 and 34)

2. Accumulation of other endogenous solutes (e.g., glucose, BUN) also can produce hyperosmolality (Cases 15, 20, 22).

3. Hypertonicity, an increase in effective osmoles (e.g., Na^+, glucose, ethylene glycol), causes water to shift from the ICF to the ECF.

a. Shift of water to the ECF causes cellular shrinkage.

b. Dehydration may be masked and hypovolemia may not be evident on physical examination because of these internal water shifts.

c. Rapid return of hypertonic ECF to isotonicity causes cellular edema, which may have dire consequences. For example, cerebral cellular edema can produce damage to the brain that leads to death.

4. Not all instances of hyperosmolality produce hypertonicity. Increased BUN concentration does not increase tonicity or produce ICF to ECF water shifts because BUN is freely diffusible across membranes.

5. Hypertonic rumen contents from urea toxicity, propylene glycol toxicity, and grain overload can produce a rapid shift of water from the ECF into the rumen. (In this situation, urea is an effective osmole, because it does not readily cross the thick epithelium of the rumen into plasma.) Secondary hypernatremic hyperosmolality may develop due to the rapid decrease in ECF water without loss of sodium.

H. Hypo-osmolality

1. Hypo-osmolality always is associated with hyponatremia (Case 8), but not all cases of hyponatremia are hypo-osmolal (e.g., when associated with hyperglycemia) (Cases 8, 15, 20, 22).

2. Hypo-osmolality produces hypotonicity, causing shifts of ECF water into the ICF with subsequent cellular swelling.

3. Rapid development of hypo-osmolality can produce intravascular hemolysis and neurologic disorders.

4. In dehydration accompanied by hypo-osmolality, loss of ECF volume is amplified by movement of water into the ICF. Therefore, circulatory collapse and shock may occur.

DETERMINATION OF BLOOD GASES AND ELECTROLYTES

I. BLOOD GAS ANALYSIS

Blood gas analysis directly measures the partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), and hydrogen ion concentration (pH). The actual blood bicarbonate (HCO_3^-), standard HCO_3^-, and base excess (BE) values (mmol/L) are calculated from the measured values.

A. Instrumentation

1. Laboratory-based blood gas instruments measure blood gases by ion-selective electrodes.

2. Blood gas analysis also can be performed on hand-held instruments that can be used at the patient's side. Manufacturers' instructions for sample handling and quality control must be followed.

B. Sample management

1. Arterial vs. venous samples. Only arterial blood samples are suitable for PO_2 determinations. Either arterial or venous samples are adequate for pH, HCO_3^-, and PCO_2 measurements, but reference intervals differ. It is important to use the appropriate reference intervals based upon the type of sample (arterial vs. venous).

2. The sample should be collected from a large, free-flowing vessel and not exposed to air before measurement.

www.Ebook777.com

3. Blood should be collected in a syringe with 0.1 to 0.2 ml heparin (1,000 U/mL). The syringe should be covered with a tight-fitting cap and delivered to the laboratory or analyzed by a point of care instrument as soon as possible.
4. Arterial samples should be analyzed immediately for accurate PO_2 determination. Analysis can be delayed up to 30 minutes, if samples are collected in glass syringes and kept on ice. Oxygen diffuses from room air through most plastic, producing falsely high results.
5. Blood pH and PCO_2 are more stable in samples than PO_2 and can be measured within 30 minutes, if samples are kept on ice.
6. Blood gas machines measure the blood sample at 37°C. Actual *in vivo* partial pressure of gas is affected by body temperature. Therefore, the patient's body temperature should be taken and reported to the laboratory or entered into the point of care instrument when a blood sample is submitted. Knowledge of the patient's body temperature allows the laboratory to make the required corrections in test values.

C. Arterial partial pressure of oxygen (PO_2)
1. PO_2 can be used to determine the amount of O_2 dissolved in plasma using the following formula:

$$O_2(\text{mEq/L}) = 0.01014 \times PO_2.$$

2. PO_2 does not reflect the total O_2 carried in the blood. Most O_2 is combined with hemoglobin and does not contribute to PO_2.
3. The total O_2 concentration depends on total hemoglobin, O_2-carrying capability of hemoglobin, body temperature, blood pH, erythrocytic 2,3-diphosphoglycerate concentration, and PO_2. The PO_2 influences the percent saturation of hemoglobin with oxygen.
4. High PO_2 can occur only when an animal is given gases with high O_2 content (e.g., via oxygen cage or anesthetic machine).
5. Low PO_2 (hypoxemia) can occur in respiratory disorders or with derangement of the respiratory control mechanisms (Case 24).

D. Partial pressure of carbon dioxide (PCO_2)
1. PCO_2 is proportional to dissolved CO_2 in the plasma.
2. Dissolved CO_2 is in equilibrium with carbonic acid (H_2CO_3) as expressed by the formula

$$H_2CO_3\ (\text{mmol/L}) = 0.03 \times PCO_2.$$

3. PCO_2 is a measure of alveolar ventilation. Decreased alveolar ventilation increases PCO_2 (hypercapnia, also called hypercarbia) (Case 23), whereas increased ventilation decreases PCO_2(hypocapnia, also called hypocarbia).

E. Hydrogen ion concentration (pH)
1. Blood pH is maintained within narrow limits in health by proteinic, phosphate, and bicarbonate buffer systems. The bicarbonate buffer system is the only one of these buffer systems that is measured for clinical evaluation of the patient.
2. Decrease in blood pH is acidemia. The condition producing this change is acidosis (Case 18).
3. Increase in blood pH is alkalemia. The condition producing this change is alkalosis (Cases 22, 23, 24).

F. Bicarbonate ion (HCO_3^-)
1. HCO_3^- concentration is calculated from the pH and PCO_2 by the Henderson-Hasselbalch equation:

$$pH = 6.1 + \log[HCO_3^- \div (0.03 \times PCO_2)];\ \text{where } 6.1 = \text{pK of carbonic acid.}$$

2. HCO_3^- concentration is maintained in health by the conservation and production of $NaHCO_3$ by the renal tubules.

Free ebooks ==> www.Ebook777.com

3. Blood gas analyzers report two calculations of HCO_3^- called the actual and standard HCO_3^- values.
   a. Actual HCO_3^- is the value calculated from the Henderson-Hasselbalch equation.
   b. Standard HCO_3^- is derived from actual HCO_3^-. It is based on the expected HCO_3^- of healthy people, the concentration of HCO_3^- if the plasma were exposed to a PCO_2 of 40 mm Hg, and the presumed normal hemoglobin concentration of the blood.
   c. Standard HCO_3^- has questionable validity in domestic animals because of interspecies variation in PCO_2 and HCO_3^-.

G. Base Excess
1. Many blood gas analyzers calculate base excess (BE), based on an expected value for blood from a healthy person having a BE of 0 ± 2.
2. Standard HCO_3^-, measured hemoglobin, PCO_2, and body temperature are used for BE calculations.
3. BE is the mEq/L of strong base or acid added to the sample that would produce a pH of 7.40 at 37°C and PCO_2 of 40 mmHg.
4. BE reflects metabolic acid-base disorders. BE greater than 0 indicates metabolic alkalosis and BE below 0 indicates metabolic acidosis.
5. The usefulness of BE as an indicator of metabolic acid-base status has been questioned for both humans and animals.
6. In health, BE varies among domestic animals. It is usually above 0 in horses and ruminants and below 0 for dogs and cats.
7. BE is often used to calculate the total body deficit or excess of HCO_3^-, but the results should be considered an estimate rather than an exact determination.
8. For most animal samples, BE and standard HCO_3^- add little information compared to actual HCO_3^- in determining appropriate fluid therapy in animals.

II. TOTAL CO_2 CONTENT (TCO_2)

TCO_2 is another way of measuring plasma HCO_3^-. TCO_2 is the total CO_2 gas released when a serum or plasma sample is mixed with a strong acid.

A. Measurement
1. TCO_2 can be measured in serum or heparinized plasma by enzymatic or ion selective electrode techniques. It is stable in samples handled for routine clinical chemistry.
2. Blood samples for TCO_2 should completely fill the sample tube. Collection of small volume samples in large-volume tubes can produce falsely low test results due to diffusion into surrounding air.
3. TCO_2 can be calculated from blood gas data, and is reported by some blood gas analyzers.
4. Severe muscle damage may cause falsely high TCO_2 as measured by enzymatic technique. TCO_2 measured by ion selective electrode or blood gas analysis is unaffected and can be used if factitiously high TCO_2 is suspected in an animal with extensive muscle damage.

B. Components of TCO_2
1. HCO_3^- is the major contributor to TCO_2. Therefore, changes in TCO_2 concentration are interpreted as changes in HCO_3^- (Cases 6, 9, 15, 18, 20, 22, 23, 24).
2. Small amounts of TCO_2 come from dissolved H_2CO_3 and carbamino acids.
3. In health, TCO_2 is approximately 1.5 mmol/L higher than HCO_3^- from the same sample, due primarily to the contribution of H_2CO_3.

III. PULSE OXIMETRY

Pulse oximeters provide rapid, non-invasive evaluation of oxygen saturation of arterial hemoglobin (SaO_2).

www.Ebook777.com

A. SaO_2 is proportional to arterial PO_2 (PaO_2). It is an indirect measure of arterial oxygenation.

B. Oximeter probes designed for people can be attached to various body sites of domestic animals to provide estimated oxygen saturation (SpO_2).

C. Pulse oximeters report SpO_2 by comparing pulsatile changes in relative absorption of light from oxygenated and reduced hemoglobin.

D. Different probes designed for human use result in variation in results from pulse oximetry, as well as different rates of failure to produce readings.

E. Pulse oximetry has been found to be reliable in horses and dogs, but not in cats.

F. Oximetry is not a replacement for blood gas analysis, but it can be a useful technique to monitor animals during surgery or other procedures requiring anesthesia.

IV. ACID-BASE REGULATION

A. Ratio of HCO_3^-/H_2CO_3 ($HCO_3^- \div [0.03 \times PCO_2]$)

1. The ratio is about 20:1 in most species (slightly less in the dog and cat) in health.

a. Decreases in the ratio cause acidemia and denote acidosis, while increases in the ratio cause alkalemia and denote alkalosis.

b. The ratio, not individual concentrations, determines pH.

2. If either HCO_3^- or H_2CO_3 increases or decreases, normal homeostatic mechanisms cause changes in the other component. The net effect is to return the ratio to 20:1.

a. Changes in HCO_3^- (called metabolic acidosis or metabolic alkalosis) produce respiratory compensation (changes in PCO_2) within minutes. Fluid therapy may be appropriate in ultimately resolving the acid-base disturbance.

b. Changes in H_2CO_3 (called respiratory acidosis or respiratory alkalosis) produce metabolic compensation (changes in HCO_3^-) after a much longer period, often several days. In clinical practice, H_2CO_3 concentration is assessed indirectly as PCO_2. Ventilatory assistance or oxygen therapy may be part of the treatment.

3. Compensation is an active physiologic process. Lesions in the respiratory or renal system interfere with normal compensation and potentiate the seriousness of the acid-base disorder.

4. Modulation of production of small organic acids, such as lactic acid and β-hydroxybutyric acid, is another method of physiologic compensation for fluctuations in body pH (Chapter 6).

a. Lactic acid or β-hydroxybutyric acid production increases rapidly in response to either respiratory or metabolic alkalosis.

b. Increased concentration of these organic acids modulates pH increase by titrating the HCO_3^- in plasma.

c. Conversely, acidosis leads to a decrease in production of small organic acids.

d. The relative importance of this mechanism for pH control in disease states of animals is not yet known.

5. Serum electrolyte values are needed to fully evaluate the cause of blood gas, pH, and HCO_3^- abnormalities as well as to determine appropriate therapy.

B. Patterns of acid-base abnormalities (Table 5.1)

1. The common types of imbalance and laboratory differentiation are shown in Table 5.1. Note that compensation produces unidirectional change in the components of the buffer to restore the HCO_3^-/H_2CO_3 ratio (e.g., if HCO_3^- is low, the compensatory change is a decrease in PCO_2).

2. Overcompensation does not occur. Shifts in HCO_3^- and PCO_2 in opposite directions indicate mixed respiratory and metabolic disorders (see Disorders of Respiratory Function).

Free ebooks ==> www.Ebook777.com

TABLE 5.1.
LABORATORY DIFFERENTIATION OF ACID-BASE IMBALANCE.

Condition	Blood pH	PCO_2	HCO_3[a]	HCO_3/H_2CO_3 ratio[b]
Metabolic acidosis	↓↓[c]	N	↓	↓↓
Uncompensated				
Partial compensation[d]	↓	↓	↓	↓
Respiratory acidosis	↓↓	↑	N	↓↓
Uncompensated				
Partial compensation[e]	↓	↑	↑	↓
Metabolic alkalosis	↑↑	N	↑	↑↑
Uncompensated				
Partial compensation[d]	↑	↑	↑	↑
Respiratory alkalosis	↑↑	↓	N	↑↑
Uncompensated				
Partial compensation[e]	↑	↓	↓	↑

[a]Total CO_2 (TCO_2) can be used as a close estimate of HCO_3 concentration.
[b]H_2CO_3 concentration = $0.03 \times PCO_2$; HCO_3/H_2CO_3 is approximately equal to 20:1 in health.
[c]Arrows indicate direction of change from reference interval: ↑↑ or ↓↓ = marked change, ↑ or ↓ = mild to moderate change, N = no change.
[d]Respiratory compensation occurs within hours of onset of metabolic disorder.
[e]Metabolic compensation may not occur for days after onset of respiratory disorder.

V. ELECTROLYTES AND ANION GAP

The electrolytes measured in most clinical situations are Na^+, K^+, Cl^-, and HCO_3^- as either HCO_3^- from blood gas analysis or as TCO_2.

A. Methods of measurement

1. Sample management

a. Serum is the best sample for electrolyte analysis. Heparinized plasma also can be used.

b. Serum should be separated from the clot as quickly as possible and kept in an airtight container to prevent *in vitro* alteration.

c. Laboratories measure electrolytes either with or without prior dilution, techniques that give slightly different results. Oftentimes, they do not state which technique is used, but should provide that information on request.

2. Measurement techniques using undiluted samples:

a. Electrolytes are present only in the aqueous phase of serum, which constitutes about 96% of serum volume in health. Electrolytes are excluded from the portion of serum comprised of proteins and lipoproteins.

b. Measurement techniques that use undiluted samples give results as mmol/L of serum water, whereas techniques using diluted samples give results as mmol/L of serum.

c. Values for healthy animals derived from techniques using undiluted samples are about 1.04-fold greater than those of other techniques.

d. Reference intervals are higher (especially for Na^+ and Cl^-) from laboratories using these techniques.

e. Increases in the non-aqueous phase (lipemia and hyperproteinemia) of serum does not affect the test results (however, test results will be affected using diluted samples).

www.Ebook777.com

f. Certain ion selective electrode (ISE) instruments use undiluted samples.
(1) Many electrolytes can be measured, including Na^+, K^+, Cl^-, TCO_2, and ionized Ca^{++} (for sample handling of the latter, see Chapter 11).
(2) Measurement is based on electrical potential across a membrane designed to be selectively sensitive to a certain electrolyte.
(3) Machines are available to measure multiple electrolytes in a single sample using a series of electrodes (e.g., Na^+, K^+, Cl^-, and TCO_2).

g. A point of care instrument that performs blood gas analysis and measures electrolyte concentrations can be used on the clinic floor. It determines electrolyte concentrations in undiluted samples by ISE techniques.

3. Techniques using diluted samples
a. Techniques using diluted samples measure electrolytes per volume of serum, including the proteins and lipoprotein non-aqueous fractions of serum.
b. Reference intervals for these techniques are lower than those for techniques using undiluted samples.
c. Falsely low values can occur in serum samples with an increased non-aqueous phase (e.g., lipemia, increased protein concentration).
d. Many instruments that measure electrolytes use diluted samples:
(1) Ion-specific electrodes. Many instruments using ISE technology use diluted samples.
(2) Flame photometry. Na^+ and K^+ concentration can be measured by the intensity of light emitted when the sample is burned in a propane flame. An internal lithium standard is used for calibration of the instrument.
(3) Cl^- can be measured by ISE, coulombmetric techniques, and colorimetric methods. Bromine toxicosis or treatment with potasssium bromide (e.g., for seizures) may causes falsely high Cl^- results by either ISE or colorimetric methods, but not by coulombmetric methods.

B. Anion gap calculations
1. The anion gap (AG) is a calculated value

$$([Na^+ + K^+] - [Cl^- + HCO_3^-])$$

that can aid in determining the cause of acid-base abnormalities. Total serum cations equal those commonly measured ($Na^+ + K^+$) plus all remaining unmeasured cations (UC). Total serum anions equal those commonly measured ($Cl^- + HCO_3^-$) plus all remaining unmeasured anions (UA) (Figure 5.1).

2. By the law of electrical neutrality,
a. Total cations = total anions.
b. $Na^+ + K^+ + UC = Cl^- + HCO_3^- + UA$.

3. Rearrangement of the above equations yields the following:
a. $Na^+ + K^+ - Cl^- - HCO_3^- = UA - UC$.
b. AG = UA − UC.

4. Frequently, TCO_2 is used to calculate AG.
a. $AG = ([Na^+ + K^+] - [Cl^- + TCO_2])$.

5. AG calculated using HCO_3^- is slightly larger than AG calculated using TCO_2. Therefore, each calculation requires its own reference interval.

C. Interpretation of the anion gap
1. In health, the major anionic components of the AG are albumin (an anion at physiologic pH), phosphates, sulfates, and small organic acids. The major cationic components are ionized Ca^{++}, Mg^{++}, and some γ-globulins (cations at physiologic pH). Some antibiotics are cations and contribute to the UC when present in large amounts.

Free ebooks ==> www.Ebook777.com

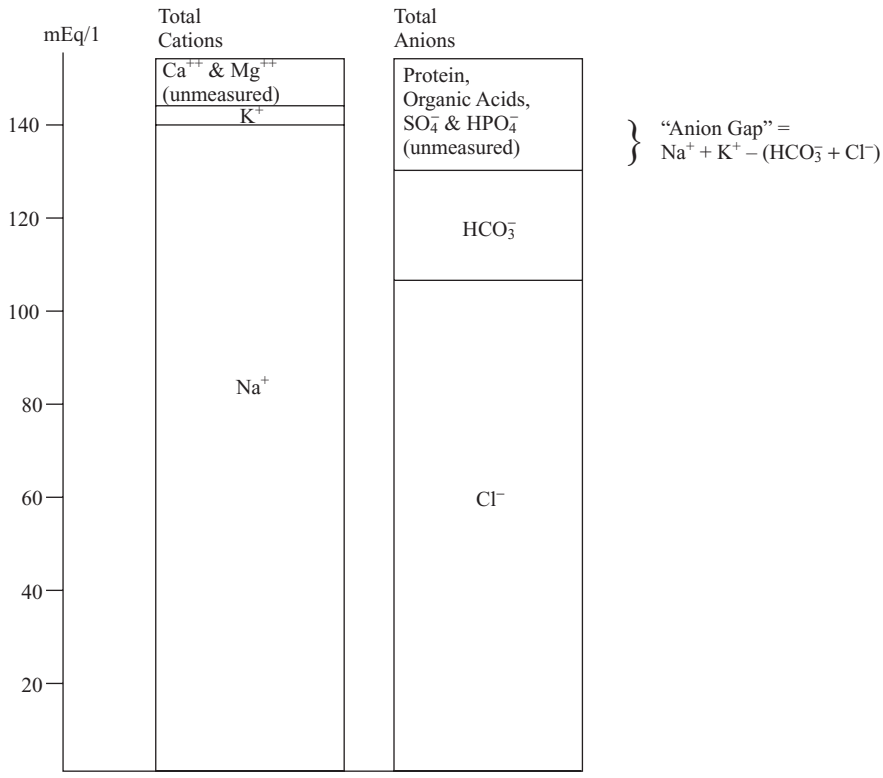


FIGURE 5.1. The ionic composition of serum. Notice that the anion gap equals unmeasured anions minus unmeasured cations and that total anions equal total cations.

2. The UC remain quite constant in health and disease. Most changes in AG occur because of changes in UA, such as small organic acids, inorganic phosphate, albumin, and exogenous toxins.

3. Increased AG occurs in many diseases, including lactic acidosis (lactate), diabetic ketoacidosis (acetoacetate, β-hydroxybutyrate), renal insufficiency (salts of uremic acids), and certain toxicities (metabolites of ethylene glycol) (Cases 15, 18, 20, 21, 22, 24, 34). A false high anion gap may occur with *in vitro* loss of HCO_3^- caused by improper sample handling.

4. Decreased anion gap is uncommon. Causes include hemodilution, hypoalbuminemia, and an increase in certain cations (e.g., hypercalcemia).

5. Albumin is a major contributor to the AG and can influence the gap in two ways.

- **a.** Hyperalbuminemia increases AG; hypoalbuminemia decreases AG.
- **b.** The number of H^+ ions bound to albumin changes with blood pH.
  - **(1)** Alkalemia causes a small decrease in albumin-bound H^+, increasing the AG slightly.
  - **(2)** Acidemia increases albumin bound H^+, decreasing the AG slightly.
- **c.** Albumin-mediated changes in AG are primarily due to changes in albumin concentration, rather than pH-mediated shifts in bound H^+. In most instances, the influence on the AG gap is mild, but can produce difficulties in its interpretation (e.g., an animal with lactic acidosis and hypoalbuminemia may have a normal AG).

6. AG should be considered an aid in interpreting electrolyte data, not a precise measure of the amount of salts of unmeasured acids in circulation.

www.Ebook777.com

ACID-BASE INTERPRETATION BASED ON STRONG ION DIFFERENCE: STEWART'S SID THEORY

In 1983, P.A. Stewart proposed an alternative theory of acid-base regulation. This theory was based on the difference in strong anions and cations as an independent influence on blood pH. This approach differs from classical acid-base interpretation. It is often referred to as the "strong ion difference" (SID) approach to acid-base interpretation. According to SID theory, blood HCO_3^- is a dependent variable, influenced by other factors.

I. BASIS OF BLOOD pH ACCORDING TO SID THEORY:

A. PCO_2 (which is converted to H_2CO_3)

B. Total weak acids [A_{tot}], primarily weak acid moieties on plasma proteins and phosphates

C. Strong ion difference (SID). Total SID is the difference between all cations and anions that completely dissociate at body pH. It includes inorganic ions, such as Na^+, K^+, Cl^- Ca^{++}, Mg^{++}, and organic ions of acids that completely disassociate at body pH, such as lactate, β-hydroxybutyric acid, and acetoacetic acid.

D. Increased SID is associated with metabolic alkalosis and decreased SID with metabolic acidosis.

II. LIMITATIONS OF SID THEORY

A. Calculation of all the independent variables requires complicated equations that are best handled by computers.

B. The equations developed for human plasma cannot be transferred to animal plasma until the constants used to determine [A_{tot}] are determined experimentally.

III. SIMPLIFIED SID THEORIES

A. Simplified SID calculations have been proposed to allow SID-based evaluation without the need for complicated calculations.

B. Multiple authors have proposed various simplified calculations of SID, so that the acronym SID does not have a single definition.

IV. USEFULNESS OF SID THEORY

A. Evaluation of TCO_2, serum electrolytes, and AG usually suffices for interpretation of most metabolic acid-base abnormalities in domestic animals.

B. The SID approach to evaluation of acid-base disorders is most helpful when multiple disorders occur together, such as mixed metabolic acid-base disorders or acid-base disorders in a hypoproteinemic patient.

VARIATIONS OF BLOOD pH AND ELECTROLYTE CONCENTRATIONS

I. CAUSES OF VARIATION IN BLOOD pH AND ELECTROLYTE CONCENTRATIONS IN HEALTHY ANIMALS

A. Postprandrial increase in blood pH.

Free ebooks ==> www.Ebook777.com

1. In monogastric animals, ingestion of food leads to a transient but slight increase in blood pH.
2. Concomitantly, urine becomes alkaline (postprandrial alkaline tide).
3. Production and secretion of gastric HCl leads to an increase in plasma $NaHCO_3$ that is rapidly excreted by the kidneys, raising urine pH.
4. The magnitude of increase is greater in older dogs compared to younger ones.

B. Alteration of ruminant blood pH by manipulation of dietary electrolytes.
1. Typical bovine diets have a net alkalinizing effect on the body, with a resultant slight increase in blood pH and production of alkaline urine.
2. Diets can be acidified by increasing the number of strong inorganic anions in the diet in relation to the number of strong inorganic cations.

C. Lowering blood pH, just prior to parturition, increases blood ionized Ca^{++} and reduces the incidence of hypocalcemic postparturient paresis (milk fever) in cows.

D. The dietary cation anion difference (DCAD) is a calculation that quantifies the important ions that determine the alkalinizing or acidifying affects of a diet. One commonly used formula is:

$$DCAD = Na^+ + K - Cl - SO_4^- \text{ (mEq/kg dry weight matter)}.$$

E. Effect of DCAD on blood and urine pH:
1. DCAD greater than 0 produces a slight metabolic alkalosis and alkaline urine (pH 8 to 9).
2. DCAD below 0 produces a slight decrease in blood pH and the production of acid urine.
3. Diets with DCAD below -50 produce mild acidosis with urinary pH of about 6. These diets have been shown to prevent milk fever.
4. Negative DCAD diets are fed to cows during late pregnancy. After calving, the cows are returned to a high DCAD diet.
5. Measurement of urine pH is used to monitor the effectiveness of the dietary acidifying regimen for dry cows.

II. INFLUENCE OF EXERCISE ON ACID-BASE BALANCE AND ELECTROLYTES

A. During intense, short-term exercise, two opposing changes can occur
1. Lactic acid production by hypoxic muscles can lead to systemic lactic acidosis.
2. Hyperventilation decreases PCO_2, increasing blood pH by hypocapnia.

B. The final blood pH depends on the balance of lactic acid production vs. hypocapnia.

C. Racing Greyhounds have moderate to marked acidemia and hypocapnia, with marked decrease in both HCO_3^- and PCO_2 after an intense sprint.

D. Labrador Retrievers develop respiratory alkalosis with little metabolic change after exercise that includes repeated retrievals of an object.

E. Endurance exercise produces changes in Na^+, K^+, and Cl^-.
1. Horses used in endurance events may become hypokalemic, hypochloridemic, and slightly hyponatremic after one to two days of continuous exercise.
2. Equine sweat has higher concentrations of Cl^- and K^+ and approximately equal concentrations of Na^+ compared to plasma.
3. Mild changes occur in all horses used in endurance races. More severe electrolyte and acid-base changes are seen in horses that develop "exhaustion syndrome."
4. Racing dogs develop hyponatremia and hypokalemia, although they do not sweat. The exact routes of their excess electrolyte loss are not known.

www.Ebook777.com

DISORDERS OF ELECTROLYTES AND METABOLIC ACID BASE IMBALANCE

It is best to have complete electrolyte profiles (Na^+, K^+, Cl^-), some measure of blood acid-base status (blood gas analysis or TCO_2), and calculation of anion gap (AG). Incomplete data may give false information about the status of the patient, leading to inappropriate treatment. Electrolyte and acid-base profiles are used primarily to assess the severity of body fluid disorders rather than to make a specific diagnosis. Sometimes these profiles can be helpful in substantiating a diagnosis. Rarely, an electrolyte pattern may be characteristic of a specific disease.

I. SERUM SODIUM

A. Physiologic considerations

1. Na^+ maintains ECF osmolality and is essential for renal water retention, a control mechanism for hydration status.

2. Essentially all Na^+ is in the ECF, making ECF Na^+ an estimate of total body Na^+.

3. Serum Na^+ (mmol/L) can be used as a measure of total body Na^+ if ECF volume (hydration status) is considered.

4. Total ECF Na^+ (mmol) = serum Na^+ (mmol/L) × ECF volume (L).

B. Hyponatremia, normonatremia, or hypernatremia may occur with low, normal, or high ECF volume (Table 5.2).

TABLE 5.2.
MECHANISMS AND DISEASES ASSOCIATED WITH VARIOUS COMBINATIONS OF SERUM NA^+ CONCENTRATION AND HYDRATION STATUS.

Hyponatremia
- Decreased ECF H_2O (hypotonic dehydration)
  - Loss of Na^+-rich fluids
    - Diarrhea (foals and horses, occasionally dogs)
    - Hypoaldosteronism
    - Osmotic diuresis (diabetes mellitus)
    - Renal disease (cattle)
    - Salmonellosis (calves)
- Normal ECF H_2O (normal hydration)
  - Dietary salt deficiency (cattle)
  - Early hypoaldosteronism
  - Early renal disease (cattle)
  - Loss of Na^+ rich fluid and treatment with low Na^+ fluids (e.g., diarrhea, blood loss treated with 5% dextrose solution)
  - Psychogenic polydipsia
  - Rapidly occurring hyperglycemia (water shift from ICF to ECF due to ECF hypertonicity)
  - Ruptured urinary bladder (foal, cow, dog)
  - Saliva loss (horse)
  - Sustained exercise (horse, dog)
- Increased ECF H_2O
  - Rapidly developing edema; hydroperitoneum (ascites) and/or hydrothorax
  - Repeated drainage of chylothorax (dog)
  - Treatment during oligouric renal failure with excessive low Na^+ fluids

Free ebooks ==> www.Ebook777.com

TABLE 5.2
CONTINUED.

- Normonatremia
  - Decreased ECF H_20 (isotonic dehydration)
    - Loss of isonatremic fluids
      - Diarrhea
      - Exudation
      - Gut fluid sequestration
      - Hemorrhage
      - Renal disease
      - Vomiting
  - Normal ECF H_20 (normal hydration)
    - Health
    - Disease without ECF and Na^+ abnormalities
  - Increased ECF H_20 (edema, hydroperitoneum, hydrothorax)
    - Cardiac failure
    - Hypoalbuminemia
    - Liver disease
    - Retention of water
    - Treatment with isonatremic fluids during above diseases
- Hypernatremia
  - Decreased ECF H_20 (hypertonic dehydration)
    - Adipisia due to abnormal thirst mechanism (neurologic disease, damage to hypothalamic osmoreceptors)
    - Adipsia due to lack of water
    - Diabetes insipidus with restricted access to water
    - Severe insensible H_20 loss (panting, sweating)
  - Normal ECF H_20 (normal hydration)
    - With normovolemia
      - Osmoreceptor defect
      - Primary hyperaldosteronism (dogs)
      - Access to high salt diet with restricted water intake (salt poisoning)
      - Consumption of seawater
      - Force feeding improperly formulated diet by pharyngostomy tube with inadequate water intake
    - With hypovolemia and increased gastrointestinal or transcellular H_2O
      - Shift of water into gastrointestinal tract (ruminants)
        - Grain overload-lactic acidosis
        - Propylene glycol toxicosis
        - Urea toxicosis (cattle)
        - Shift of water into third space (bile peritonitis)
  - Increased ECF H_20 (edema, ascites, hydrothorax)
    - Rare situation: Treatment with hypernatremic fluids during renal shutdown

1. Normonatremia (serum Na^+ within reference interval) can occur with normal, decreased or increased total body Na^+. For example:

a. Normomatremia in acute blood loss represents decreased total body Na^+: the patient has lost both Na^+ and water in physiologic proportions.

b. Normonatremia in the presence of ascites or edema represents increased total body Na^+: the increase in ECF water has been matched by increase in Na^+.

www.Ebook777.com

2. Hyponatremia or hypernatremia occur when Na^+ losses or gains do not match ECF volume losses or gains. They can be present with low, normal or high ECF volume. For example:

a. Hyponatremia in a dehydrated animal is due to loss of both Na^+ and water, with Na^+ losses being greater than water losses. The result is severe decrease of total body Na^+.

b. Hyponatremia in an animal with edema or ascites is due to water accumulation greater than Na^+ increases. The total body Na^+ is normal to increased.

c. Hypernatremia in a dehydrated animal is due to loss of water without loss of Na^+: Total body Na^+ is normal, but ECF volume is decreased.

d. Hypernatremia in an animal with normal ECF volume is due to increased total body Na^+ (usually excess intake of salt without access to water).

C. The mechanisms and diseases responsible for each possibility are given in Table 5.2.

D. Evaluation of the Na^+/K^+ ratio has been advocated as a method of recognizing hypoaldosteronism, but has some important limitations.

1. Hypoaldosteronism (Addison's disease) is associated with a calculated Na^+/K^+ ratio less than 24.

2. Many other conditions may produce similar Na^+/K^+ ratios, including the following:

a. Renal disease

b. Gastroenteritis treated with low Na^+ fluids

c. Diarrhea caused by *Trichuris vulpis* infection

d. Repeated drainage of chylothorax

e. Large volume peritonitis and pleuritis

f. Illness during late-term pregnancy

3. The probability of hypoaldosteronism increases as the Na^+/K^+ ratio decreases. A ratio less than 19 is highly associated with, but is not specific for, hypoaldosteronism.

E. The Na^+/K^+ ratio should be considered along with presence or absence of hyponatremia and hyperkalemia when evaluating the electrolyte status of a patient.

II. SERUM POTASSIUM

A. Physiologic considerations

1. Serum K^+ is maintained within narrow limits for normal neuromuscular and cardiac function.

2. Serum K^+ is not a reliable indicator of total body K^+ because most ion concentration is in the ICF. Serum K^+ can be altered by the following:

a. Shifts of K^+ between the ICF and ECF (internal K^+ balance).

b. Increases and decreases in total body K^+ (external K^+ balance).

c. Mixed disorders of both internal and external types.

3. Species vary in the amount of intra-erythrocytic potassium.

a. The erythrocytes of horses, pigs, and primates are high in K^+.

b. Intra-erythrocytic K^+ concentration varies from moderate to low in certain breeding groups of cattle and sheep.

c. Intra-erythrocytic K^+ concentration is low in most cats and dogs, except:

(1) Erythrocytes of Akitas, Shiba Inus, and some other Japanese breeds of dogs have a high intracellular K^+ concentration.

(2) Occasionally, dogs of other breeds or mongrels have inherited high K^+ erythrocytes.

4. Reticulocytes are higher in K^+ compared to mature erythrocytes.

5. Sources of erroneously high serum K^+ from sampling error (pseudohyperkalemia) include the following:

a. Serum K^+ is slightly higher than plasma K^+ of healthy animals due to release of K^+ from platelets during clotting.

Free ebooks ==> www.Ebook777.com

b. Marked thrombocytosis may produce markedly increased serum K^+; measurement of K^+ in heparinized plasma reduces this source of error.

c. Leakage from high K^+ erythrocytes. Hemolysis or prolonged contact between the sample's serum and clot may produce factitious hyperkalemia, especially in those species or situations with high K^+ erythroctyes. The hemolysis may be covert; although the serum or plasma is not discolored, K^+ concentration is significantly increased.

6. Because serum K^+ is affected by both internal and external K^+ balance or a combination of the two, both possibilities should be considered when determining proper K^+ therapy.

B. Mechanisms and causes of hyperkalemia (Table 5.3). Hyperkalemia can lead to life-threatening cardiac conduction abnormalities, bradycardia, and electrocardiogram changes.

1. Hyperkalemia secondary to changes in external K^+ balance may be due to the following:

a. Decreased urinary excretion

(1) Normally, most excess K^+ is excreted in urine (kaliuresis).

(2) Anuric or oliguric renal disease, postrenal blockage (feline urologic syndrome), or ruptured bladder (foals) can produce hyperkalemia (Cases 18, 20).

(3) Dogs and ruminants usually do not have hyperkalemia with postrenal obstruction or ruptured bladder.

b. Hypoaldosteronism

(1) Aldosterone is one of the mediators of renal K^+ excretion.

(2) Hypoaldosteronism (Addison's disease) is characterized by hyperkalemia and hyponatremia.

c. Increase in body cavity fluid volume may produce hyperkalemia and hyponatremia (dogs and cats).

(1) Repeated drainage of chylothorax causes retention of K^+ and can result in hyperkalemia by unknown mechanisms (dogs, clinical and experimental data).

(2) Peritoneal effusion in cats may cause hyperkalemia and hyponatremia.

d. Hyperkalemia and hyponatremia have been seen in ill bitches during late pregnancy.

e. High doses of trimethoprim-sulfamethoxazole decrease K^+ excretion by inhibiting an enzyme needed for distal renal tubular Na^+-K^+ exchange.

f. Parenteral administration of K^+

(1) Rapid administration of therapeutic K^+ may produce life-threatening hyperkalemia. In general, large animals are less susceptible to this problem than small animals.

(2) Oral K^+ supplementation can be used in many conditions with less danger to the patient.

2. Hyperkalemia, secondary to changes in internal K^+ balance, can occur from the following:

a. Some instances of acidemia:

(1) Hyperkalemia occurs in some forms of acidosis, due to ECF H^+ exchanging with ICF K^+.

(2) Hyperkalemia occurs most frequently in acidosis due to selective loss of $NaHCO_3$ and retention of Cl^- (e.g., acidosis from secretory diarrhea). ICF K^+ exchanges with excess ECF H^+ to maintain electrical neutrality. This cation exchange occurs because the acidosis is accompanied by increased plasma Cl^-, which does not readily diffuse into the ICF.

(3) Hyperkalemia is infrequent in titration (organic acid excess) acidosis. It is thought that organic acid anions enter cells with the excess H^+, maintaining electrical neutrality and eliminating the need for cation exchange.

(4) Hyperkalemia is rare with respiratory acidosis. It is thought that dissolved CO_2 diffuses freely into cells, maintaining pH equality between ECF and ICF.

(5) Rapid administration of alkalinizing fluids to acidotic patients may cause the reverse cation exchange (K^+ going to the ICF as H^+ moves to the ECF), producing life-threatening hypokalemia.

b. Hyperosmolality. Hypertonicity can produce a shift of ICF K^+ to the ECF. This shift is independent of blood pH; the mechanisms are unknown.

www.Ebook777.com

TABLE 5.3.
MECHANISMS AND CAUSES OF SERUM K^+ ABNORMALITIES.

- Hyperkalemia
  - Changes in external balance (net accumulation of ECF-K^+ from environment)
    - Anuric or oliguric renal failure
    - Postrenal obstruction (cats, foals, but not dogs, ruminants)
    - Hypoaldosteronism
    - Drainage of chylothorax (dogs)
    - Marked pleural or peritoneal effusion (dogs, cats)
    - Treatment with trimethoprim-sulfamethoxazole (dogs)
    - Hyperkalemic fluid therapy
    - Polyuric renal disease (horse)
  - Changes in internal balance (shifts from ICF-K^+ to ECF-K^+)
    - Secretory diarrhea with loss of HCO_3^- (calves, rarely dogs)
    - Insulin deficiency—diabetes mellitus (may be masked by external K^+ loss)
    - Hypertonicity
    - Marked muscle exertion (mild K^+ increase)
    - Massive tissue necrosis (saddle thrombus, rhabdomyolysis)
    - Oleander toxicity (inhibition of Na^+-K^+ ATPase)
    - Inherited hyperkalemic periodic paralysis (horses)
    - Hypthyroidism during exercise (dogs—experimental data only)
- Hypokalemia
  - Changes in external balance (decreased K^+ intake or increased K^+ loss to environment)
    - Anorexia (especially herbivores)
    - Low potassium diet
    - Loss of gastrointestinal fluids
      - Vomiting
      - Abomasal stasis and internal vomiting into rumen
      - Draining of stomach by naso-gastric tube (horse)
      - Diarrhea (especially horses)
    - Increased renal loss (kaliuresis)
      - Hyperaldosteronism (pathologic or iatrogenic)
      - Metabolic acidosis (acute)
      - Metabolic alkalosis
      - Polyuric renal disease (especially cat and cow, but not horse)
      - Renal tubular acidosis
    - Some acidifying diets (cat)
    - Profuse sweating (horse)
    - Prolonged exercise (horse, dog)
  - Changes in internal balance (shifts from ECF-K^+ to ICF-K^+)
    - Alkalemia (mild if ICF-K^+ is replete)
    - Insulin therapy
    - Rapid correction of metabolic acidosis (marked if ICF K^+ is depleted)
    - Rapid food replenishment after starvation (refeeding syndrome)

Free ebooks ==> www.Ebook777.com

c. Cell membrane damage. Loss of cell membrane integrity produces efflux of ICF K^+ to the ECF.

d. Tissue necrosis

(1) Necrosis of a large mass of tissue, especially muscle, may release a large amount of K^+ and produce hyperkalemia (e.g., saddle thrombus in cats, white muscle disease in cattle).

(2) Less severe cell damage may produce the same shifts, but restoration of normal cell function may cause rapid reversal of K^+ balance, leading to ECF K^+ depletion and hypokalemia.

e. The toxin of oleander (*Nerium oleander*) causes inhibition of cardiac Na^+-K^+ ATPase, producing hyperkalemia soon after ingestion.

f. Insulin deficiency

(1) Insulin facilitates the entry of K^+ into the ICF; therefore, deficiency of insulin may be accompanied by loss of ICF K^+ to the ECF and hyperkalemia.

(2) Diabetes causes renal loss of K^+ that can be greater than the internal shift of K^+ to the ECF.

(3) Hyperkalemia, normokalemia, or hypokalemia may occur depending on the balance of the rates of internal shifts and external losses.

(4) Administration of exogenous insulin causes rapid entry of K^+ into the ICF, and K^+ supplementation may be needed to prevent hypokalemia.

g. Inherited periodic hyperkalemic paralysis (horses) produces transient paralysis accompanied by hyperkalemia that resolves as the episode ends.

h. Exercise induces transient hyperkalemia in hypothyroid dogs due to deficiency of Na^+-K^+-ATPase concentration in muscle (experimental data).

i. Intracelluar K^+ depletion can increase the magnitude of ECF to ICF K^+ shift after the correction of the above problems. Because no clinical measure can accurately assess K^+ depletion, sufficiency of K^+ replacement should be a consideration in any condition known to be associated with external K^+ loss.

j. Many conditions that produce hyperkalemia due to internal shifts (e.g., renal tubular acidosis, severe diarrhea, diabetic ketoacidosis) also cause increased external K^+ losses. The patient may progress from hyperkalemia to normokalemia to hypokalemia, depending on the balance of internal shift and external loss.

C. Mechanisms and causes of hypokalemia (Table 5.3) include the following:

1. Hypokalemia is almost always associated with depletion of ICF K^+. Loss of K^+ can potentiate the seriousness of a disease by producing life-threatening cardiac abnormalities, skeletal muscle weakness and myopathy, loss of renal concentrating ability, and perpetuation of metabolic alkalosis (paradoxical aciduria).

2. Hypokalemia secondary to changes in external K^+ balance may be due to the following:

a. Decreased oral intake

(1) Anorectic animals (especially herbivores) may have a negative K^+ balance, especially during the two to three days needed for the kidneys to convert from K^+ excretion to K^+ conservation.

(2) Some K^+-deficient acidifying diets for cats produce hypokalemia and acidosis. These cats develop periodic hypokalemic paralysis and/or renal disease.

b. Increased gastrointestinal losses. Loss of gastric and intestinal fluids, which are rich in K^+, can produce K^+ depletion (e.g., vomiting, abomasal disorders, and diarrhea, especially severe in horses).

c. Increased urinary loss (kaliuresis). Several conditions cause this, including polyuric states (e.g., osmotic diuresis, rapid rehydration), metabolic alkalosis, acute metabolic acidosis, increased mineralocorticoid concentration, renal tubular acidosis, and diuretic therapy.

www.Ebook777.com

d. Primary hyperaldosteronism from aldosterone secreting tumors may produce marked kaliuresis with hypokalemia so severe that myopathy and profound muscle weakness can occur (cats, dogs, ferret).

3. Hypokalemia secondary to changes in internal K^+ balance may be due to the following:

a. Alkalemia (Cases 23, 24)

(1) Low ECF H^+ concentrations may cause ICF H^+ to leave cells by exchanging with ECF K^+. This internal shift in K^+ is thought to be a minor cause of hypokalemia in alkalosis.

(2) Metabolic alkalosis produces mild hypokalemia, but respiratory alkalosis does not produce these shifts.

(3) In most diseases associated with metabolic alkalosis, hypokalemia is due to decreased body K^+ from increased losses and decreased intake.

(4) Shifts of K^+ to the ICF may occur upon therapeutic alkalinization with parenteral fluids, producing life-threatening hypokalemia in a patient that was normokalemic or hyperkalemic prior to therapy.

b. ICF K^+ depletion

(1) K^+ moves more quickly from ECF to ICF if K^+ depletion has occurred.

(2) Therapy that leads to K^+ shifts into the ICF (e.g., insulin, HCO_3^-) may produce profound life-threatening hypokalemia in the K^+-depleted patient.

III. SERUM CHLORIDE

A. Physiologic considerations

1. Cl^- is the major anion of ECF.

2. Cl^- is an important component of many secretions (e.g., gastric fluid, sweat, and saliva in horses), as either NaCl, KCl, or HCl.

B. Abnormalities

1. NaCl-related changes include:

a. Increase and decrease in serum Cl^- may parallel changes in serum Na^+ concentration (Case 24).

b. Total body Cl^- varies in parallel to Na^+, and may be increased or decreased without hypochloridemia or hyperchloridemia being present if the ECF volume is increased or decreased.

2. Selective Cl^- changes include the following:

a. Cl^- may increase or decrease due to changes not related to alterations in NaCl concentration.

b. Loss of HCl- or KCl-rich secretions leads to hypochloridemia due to greater loss of Cl^- compared to Na^+ (Cases 23 and 34).

3. Loss of $NaHCO_3$-rich fluid in intestinal secretions or urine causes a relative increase in serum or plasma Cl^-, although a total body Cl^- deficit exists if dehydration is present.

C. Two calculations can be used to distinguish NaCl related abnormalities from selective Cl^- abnormalities.

1. Corrected Cl^- [$Cl^-_{(cor)}$]. When Na^+ is abnormal, Cl^- can be corrected by the following formula:

$$Cl^-_{(cor)} = Cl^-_{(measured)} \times (\text{mean } Na^+ \div \text{measured } Na^+);\ \text{mean } Na^+ = \text{mean of reference range for laboratory.}$$

a. Reference interval of $Cl^-_{(cor)}$ is approximately the same as the reference interval of measured Cl^-.

b. Evaluation of $Cl^-_{(cor)}$ to recognize selective Cl^- abnormalities is most useful when hyponatremia exists. In contrast, almost all instances of hypernatremia are due to NaCl-associated abnormalities.

Free ebooks ==> www.Ebook777.com

2. Na-Cl difference. This is another calculation to separate NaCl-mediated from selective Cl^- abnormalities. The formula is:

$$\text{Na-Cl difference} = Na^+ - Cl^-.$$

a. Interspecies variations in Na-Cl difference occur.
b. Approximate reference ranges are:
(1) Dogs and cats: 29 to 42 mmol/L.
(2) Horses: 34 to 43 mmol/L.
(3) Cattle: 35 to 45 mmol/L.
(4) Goats: 33 to 43 mmol/L.
c. The reference interval for Na-Cl difference depends on the method used to determine electrolyte concentrations. Reference intervals should be calculated by individual laboratories.

D. Selective Cl^- abnormalities are often associated with metabolic acid-base disorders
1. Selective decrease in Cl^- is almost always associated with loss of HCl-rich fluids and metabolic alkalosis (see section on metabolic alkalosis).
2. Selective increase in Cl^- may be present with metabolic acidosis due to selective loss of $NaHCO_3$ (see section on secretory acidosis).
3. Metabolic compensation for chronic respiratory disorders, an uncommon clinical situation in animals, can produce similar selective Cl^- changes due to renal retention or excretion of $NaHCO_3$.

E. Albumin concentration influences Cl^- values
1. Hyperchloridemia may occur in hypoalbuminemic animals. Albumin is an anion. When albumin-related anions are decreased, relatively more Cl is present in plasma. The AG also may be decreased (see section on anion gap).
2. Low-protein fluids (peritoneal, pleural, and pericardial fluids and CSF) have higher Cl^- reference intervals because they contain relatively little albumin.

F. Consequences of Cl^- deficiency include the following:
1. Selective Cl^- deficiency often is associated with metabolic alkalosis.
2. Chloride deficiency causes polydipsia and decreased renal concentrating ability, based upon experimental data in dogs and cattle.

IV. METABOLIC ACIDOSIS

A. Decreased plasma HCO_3^- or serum TCO_2 concentrations indicate metabolic acidosis.
1. Moderate metabolic acidosis is present when plasma HCO_3^- or serum TCO_2 concentrations are as follows:
a. 15 to 20 mmol/L in most species
b. 12 to 17 mmol/L in the dog and cat
2. Severe metabolic acidosis is present when plasma HCO_3^- or serum TCO_2 concentrations are as follows:
a. Less than 15 mmol/L in most species.
b. Less than 12 mmol/L in the dog and cat.
3. Respiratory compensation is achieved by hyperventilation to exhale CO_2 and restore the HCO_3^-/H_2CO_3 ratio.

B. Mechanisms and causes
1. HCO_3^- loss (secretory) acidosis
a. HCO_3^- can decrease when fluids rich in $NaHCO_3$ and/or $KHCO_3$ are lost from the body.
b. $NaHCO_3$ is synthesized by renal or secretory cells by rearranging the ions of $HHCO_3$ and NaCl as follows:

www.Ebook777.com

$$NaCl + HHCO_3 \rightarrow NaHCO_3 \text{ (lost to body)} + HCl \text{ (retained)}.$$

c. When $NaHCO_3$-rich fluids are lost in excess, the net effect is acidification by accumulation of HCl in the body.

d. Diseases and conditions associated with HCO_3^- loss acidosis:

(1) Saliva in animals that cannot swallow, especially ruminants (but not in the horse).

(2) Intestinal and pancreatic secretions, either trapped in the gut by obstruction or lost as diarrhea (Case 6).

(3) Urine rich in HCO_3^- in cases of renal tubular acidosis.

e. The electrolyte pattern in HCO_3^- loss acidosis (Figure 5.2)

(1) Low plasma HCO_3^- or serum TCO_2

(2) Serum Cl^- that is within the reference interval or increased accompanied by a decreased Na^+-Cl^- difference

(3) Normal anion gap

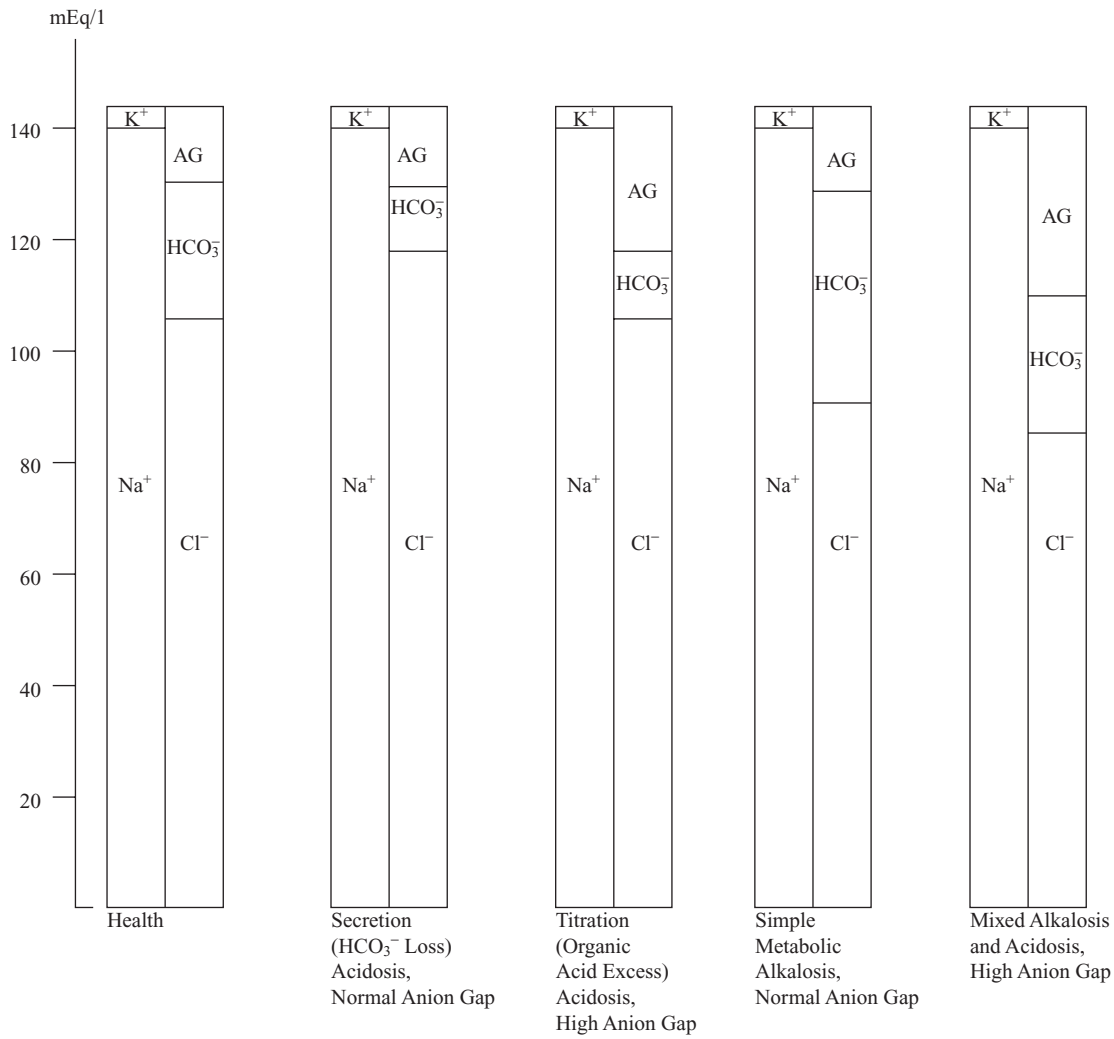


FIGURE 5.2. Electrolyte patterns in common metabolic acid-base disorders.

Free ebooks ==> www.Ebook777.com

(4) If hyponatremia exists, the measured Cl^- may be lower, but the Cl (cor) will be within the reference interval or increased.

2. Titration (organic acid excess) acidosis
   a. Organic acid accumulation can lead to HCO_3^- loss by titration.
   b. As HCO_3^- acts as a buffer, it is converted to the salt of the organic acid. For the acid HA: HA + $NaHCO_3 \rightarrow H_2C0_3$ + NaA. The HCO_3^- concentration decreases as the concentration of NaA increases.
   c. The presence of the salt of the acid (i.e., NaA in the above example) is recognized by an increased anion gap.
   d. Clinically important organic acids
      (1) L-lactic acid from mammalian anaerobic glycolysis in hypoxia and shock
      (2) D-lactic acid due to bacterial catabolism of carbohydrates in adult ruminants with grain overload, neonatal calves with diarrhea, neonatal goat kids (floppy kid syndrome), neonatal lambs and calves with neurologic abnormalities, carbohydrate-induced laminitis (horses and ponies), bacterial overgrowth secondary to pancreatic exocrine insufficiency (reported in one cat)
      (3) D-lactic acid from the glyoxalase pathway in mammalian diabetes (cats) and with high doses of propylene glycol.
      (4) Acetoacetic acid and β-hydroxybutyric acid (ketone bodies) in diabetic ketoacidosis, fat catabolism from starvation, and ketosis of ruminants (Chapter 6, Case 34).
      (5) Uremic acids in renal failure (Cases 18, 20)
      (6) Some organic poisons and their metabolites (e.g., ethylene glycol, propylene glycol, and metaldehyde).
   e. The electrolyte pattern in titration acidosis (Figure 5.2)
      (1) Low plasma HCO_3^- or serum TCO_2
      (2) Serum Cl^- and Na-Cl difference within the reference intervals
      (3) High anion gap
      (4) If hyponatremia exists, the Cl^- concentration may be low, but $Cl^-_{(cor)}$ is within the reference interval.
   f. Mixed titration and secretory acidosis can occur when dehydration from diarrhea leads to accumulation of lactic acid from hypovolemic shock. Although the acidoses from titration and secretion are both moderate, they combine to produce marked acidemia. The electrolyte pattern is as follows:
      (1) Markedly low plasma HCO_3^- or serum TCO_2 concentrations
      (2) Serum Cl^- concentration and Na-Cl difference are usually within the reference intervals, but may have mild changes typical of secretory acidosis.
      (3) Mild to moderate increase in anion gap
      (4) If hyponatremia exists, the Cl^- concentration may be low, but $Cl^-_{(cor)}$ is usually within the reference interval.

V. METABOLIC ALKALOSIS

A. Increased plasma HCO_3^- or serum TCO_2 concentrations indicate metabolic alkalosis (Figure 5.2).
   1. Moderate metabolic alkalosis is present when the plasma HCO_3^- or serum TCO_2 concentrations are as follows:
      a. 33 to 38 mmol/L in most species
      b. 27 to 32 mmol/L in the dog and cat
   2. Severe metabolic alkalosis is present when the plasma HCO_3^- or serum TCO_2 concentrations are as follows:
      a. greater than 38 mmol/L in most species
      b. greater than 32 mmol/L in the dog and cat

www.Ebook777.com

3. Respiratory compensation by hypoventilation to retain CO_2 and restore the HCO_3^-/H_2CO_3 ratio is restricted by the need for O_2. Therefore, respiratory compensation is often poor.

B. Loss of gastric or abomasal HCl is almost always the cause of metabolic alkalosis (Cases 22, 23).

1. HCl is secreted in the stomach by parietal cells by the following reaction: $NaCl + H_2CO_3 \rightarrow HCl$ (secreted) + $NaHCO_3$ (retained in body fluids). In health, the HCl is later reabsorbed in the lower gastrointestinal tract, restoring the acid-base equilibrium.
2. Vomiting in monogastric animals and drainage of gastric contents by stomach tube in horses with colic leads to loss of HCl.
3. Abomasal or high gut obstruction in ruminants leads to reflux of abomasal contents into the rumen, sometimes called "internal vomiting." Causes include physical blockage (e.g., abomasal displacement or torsion, small intestinal obstruction), functional occlusion (e.g., vagal indigestion), and stasis due to metabolic causes (e.g., renal disease, endotoxemia, hypocalcemia).
4. Proximal jejunitis-ileitis in horses produces mild metabolic alkalosis due to pooling of HCl in the stomach.
5. Loss of HCl leads to a net gain in HCO_3^-; Cl^- is lost as well as H^+, leading to alkalosis, hypochloridemia, and an anion gap that is within the reference interval or slightly increased.
6. Without laboratory evaluation, the observation of vomiting alone cannot be taken as an indication of metabolic alkalosis. With gastric reflux, vomiting may include the loss of HCO_3^-–rich pancreaticoduodenal fluid as well as gastric HCl.

C. Paradoxical aciduria in HCl loss, metabolic alkalosis (Case 23)

1. Renal correction of metabolic alkalosis should be the secretion of excess $NaHCO_3$ and retention of H^+ ions to restore the HCO_3^-/H_2CO_3 ratio. Often the kidney cannot correct the alkalosis. This condition is recognized by acidic urine concomitant with metabolic alkalosis (i.e., paradoxical aciduria).
2. Paradoxical aciduria develops as the result of hypovolemia, hypochloridemia, and total body K^+ depletion.
   a. As the kidneys retain water, they also retain Na^+ in an attempt to restore total ECF Na^+ concentration to reference intervals. Because Cl^- concentration is deficient in the glomerular filtrate, HCO_3^- is reabsorbed as the anion with Na^+.
   b. Na^+ also can be reabsorbed by exchange and secretion of H^+ or K^+. Because K^+ is deficient in the plasma, H^+ is secreted into the urine.
   c. The loss of HCO_3^- from and the addition of H^+ to the renal filtrate leads to acid urine, and the retention of HCO_3^- perpetuates the state of metabolic alkalosis.
3. Fluid therapy in cases of metabolic alkalosis with paradoxical aciduria should be directed toward correcting the NaCl deficit. Satisfactory fluids are normal saline and Ringer's solution. When K^+ depletion is severe, treatment with parenteral or oral K^+ may be necessary to reverse paradoxical aciduria.

D. Rare causes of metabolic alkalosis:

1. Hypokalemia associated with hypovolemia may lead to metabolic alkalosis. Possible clinical situations include dehydration with concomitant diuretic use or mineralocorticoid excess (either exogenous administration or endogenous release).
2. Liver failure, especially in horses, may result in the presence of excess bases (NH_3 and amines) in circulation.
3. Use of esophageal tubes for alimentation in the horse may lead to loss of saliva, which is rich in NaCl. Metabolic alkalosis with hyponatremia and hypochloridemia occur after a few days of salivary loss.
4. Exhaustion in horses used for endurance events produces a mild hypochloridemic metabolic alkalosis.

Free ebooks ==> www.Ebook777.com

5. A blow fly (*Lucilia cuprina*) of sheep produces large amounts of NH_3, resulting in hyperammonemia and metabolic alkalosis in infected animals.

E. The electrolyte pattern in hypochloridemic metabolic alkalosis:
1. Increased plasma HCO_3^- or serum TCO_2 concentrations
2. Decreased plasma or serum Cl^- concentration
3. If hyponatremia exists, selective hypochloridemia can be recognized by an increased Na-Cl difference or low $Cl^-_{(cor)}$.

VI. MIXED METABOLIC ACIDOSIS AND ALKALOSIS (CASES 22, 24)

A. Laboratory findings (Figure 5.2)
1. Plasma HCO_3^- or serum TCO_2 concentrations that are close to or within the reference interval
2. Decreased serum Cl^- concentration
3. If hyponatremia exists, selective hypochloridemia can be recognized by an increased Na-Cl difference or decreased $Cl^-_{(cor)}$.
4. Very high anion gap. Measurement of blood gases or TCO_2 alone will never detect mixed metabolic alkalosis and acidosis; the high anion gap is an important indicator.

B. Mechanisms and causes of mixed metabolic acidosis and alkalosis
1. Loss of HCl-rich fluids may lead to alkalosis and hypovolemic shock. The increased HCO_3^- from the metabolic alkalosis is titrated by the lactic acid from shock. The HCO_3^- may decline close to or within the reference interval, but the Cl^- concentration remains low while the anion gap is very high.
2. Some conditions with titration acidosis may produce vomiting (e.g., diabetic ketoacidosis, renal failure). Vomiting increases HCO_3^- from the extremely low concentrations produced by the original condition. Serum Cl^- concentration is low, and the anion gap is extremely high (often greater than 30 mmol/L).
3. Restoration of ECF volume by fluids that are low in Cl^- concentration may correct the acidosis but perpetuate the alkalosis. Fluid therapy should include sufficient Cl^- and K^+ concentrations to correct both conditions.

DISORDERS OF RESPIRATORY FUNCTION

Blood gas analysis is a relatively insensitive measure of pulmonary function and should be used as an adjunct to physical examination, radiology, and other diagnostic methods. Arterial PO_2 and PCO_2 can be helpful in assessing the severity of respiratory disorders and in monitoring respiratory status during anesthesia. Differentiation of pathologic changes in PCO_2 (respiratory acid-base imbalance) from compensation for metabolic acid-base disorders can be made only by evaluation of a full blood gas and electrolyte profile.

I. ARTERIAL PO_2

A. PO_2 is a measure of intrapulmonary gas exchange.
1. In health, regional distribution of air to areas of the lungs is balanced with regional blood flow.
2. O_2 is almost 20 times less diffusible than CO_2 across the alveolar capillary barrier. Many intrapulmonary lesions can produce hypoxemia without altering CO_2 exchange.

B. Conditions that cause hypoxemia and compensatory hyperventilation (low PO_2 accompanied by normal PCO_2 or low PCO_2, respiratory alkalosis) are caused by decreased gas exchange and concomitant continuation of CO_2 exchange (Case 24). Examples include the following:

www.Ebook777.com

TABLE 5.4.
HYPOVENTILATORY DISEASES AND CIRCUMSTANCES ASSOCIATED WITH HYPOXEMIA AND HYPERCAPNIA (HYPERCARBIA), (LOW PaO_2, HIGH $PaCO_2$), AND RESPIRATORY ACIDOSIS.

Abnormalities of neurogenic control
- Anesthesia
- Sedation
- Head trauma

Muscular or mechanical failure in breathing
- Pneumothorax
- Pleural effusion
- Ivermectin toxicity (especially in Collies and Shetland Sheep Dogs)
- Muscular weakness associated with Coonhound paralysis
- Myasthenia gravis
- Neurotoxins (botulism, tetanus)
- Paralytic drugs (succinylcholine)

Upper airway obstruction
- Calf diphtheria
- Tracheal collapse
- Laryngeal edema or constriction

Pulmonary abnormalities
- Pneumonia (severe*)
- Pulmonary edema (severe*)
- Chronic obstructive lung disease

*Less severe pneumonia and pulmonary edema may lead to hypoxemia without respiratory acidosis.

1. Perfusion/diffusion abnormalities, which include many cases of pneumonia, pulmonary edema, and pulmonary thrombosis.
2. Decreased intrapulmonary gas diffusion caused by thickened alveolar septa, pulmonary fibrosis, and pulmonary edema.

C. Conditions that cause hypoxemia accompanied by increased PCO_2 (hypoxemia and respiratory acidosis) are caused by decreased alveolar ventilation (Table 5.4).

II. ARTERIAL PCO_2

A. Arterial PCO_2 is a measure of alveolar ventilation.
1. Minute-to-minute neurologic control of PCO_2 occurs in health.
2. The aortic and carotid bodies respond to total O_2 content in chronic hypoxemia, and can become the major controllers of respiration.
3. Well-ventilated areas of the lungs can compensate for regions with poor gas exchange, causing hypoxemia with normal PCO_2 as described above.

B. Respiratory acidosis
1. Hypercapnia (hypercarbia), $PaCO_2$ above reference interval, indicates hypoventilation.
2. Hypoventilation can occur with deranged central control, failure of the mechanical apparatus of breathing, and severe pulmonary abnormalities (Table 5.4).
3. Diseases producing respiratory acidosis also produce hypoxemia because O_2 exchange is always less efficient than CO_2 exchange.

Free ebooks ==> www.Ebook777.com

TABLE 5.5.
CAUSES OF HYPERVENTILATION PRODUCING HYPOCAPNIA (HYPOCARBIA), LOW $PaCO_2$, AND RESPIRATORY ALKALOSIS.

Altered respiratory control
Convulsions
Fear or anxiety (panting)
Fever
Heat exposure
Hepatic encephalopathy
Hypoxemia (low PaO_2)
Hypotension
Pulmonary vascular shunts
Pulmonary fibrosis
Pneumonia
Pulmonary edema
Iatrogenic causes
Mechanical ventilation
Rapid correction of metabolic acidosis

4. The hypercapnia of respiratory compensation for metabolic alkalosis is blunted by the development of hypoxemia.

C. Respiratory alkalosis

1. Hypocapnia (hypocarbia), $PaCO_2$ below reference interval, indicates hyperventilation and is always associated with altered respiratory control (Table 5.5).

2. The respiratory centers may be influenced by physiologic (e.g., heat control) or pathologic (e.g., hepatic encephalopathy) stimuli (Case 12).

3. Pulmonary lesions (e.g., pneumonia) may produce hypoxemia and hypocapnia, unless diffuse, severe lung damage prevents alveolar ventilation (see above discussion on arterial PO_2).

D. Mixed respiratory and metabolic acid-base disorders

1. The laboratory findings that indicate this mixed disorder are:

a. Lack of expected compensation (e.g., normal PCO_2 and abnormal HCO_3^- or abnormal PCO_2 and normal HCO_3^-) (Case 24).

b. PCO_2 and HCO_3^- changes which produce both alkalosis and acidosis (e.g., low PCO_2 and high HCO_3^- or high PCO_2 and low HCO_3^-).

2. Respiratory compensation occurs within minutes after the onset of a blood pH abnormality. It should be expected in short-term as well as long-term metabolic disorders.

3. Metabolic compensation may lag several days after the onset of a respiratory disorder. Acute respiratory conditions may need to be re-evaluated after three to four days to determine if compensation has occurred.

4. Mixed disorders may lead to severe blood pH abnormalities; therapy should be directed toward returning the blood pH value to the reference interval.

REFERENCES

Ash RA, Harvey AM, Tasker S: 2005. Primary hyperaldosteronism in the cat: A series of 13 cases. *J Fel Med Surg* 7:173–182.

www.Ebook777.com

Bissett SA, Lamb M, Ward CR: 2001. Hyponatremia and hyperkalemia associated with peritoneal effusion in four cats. *J Am Vet Med Assoc* 218:1590–1592.

Bleul U, Schwantag S, Stocker H, et al: 2006. Floppy kid syndrome caused by D-lactic acidosis in goat kids. *J Vet Intern Med* 20:1003–1008.

Boag AK, Coe RJ, Martinez TA, et al: 2005. Acid-base and electrolyte abnormalities in dogs with gastrointestinal foreign bodies. *J Vet Intern Med* 19:816–821.

Collins ND, LeRoy BE, Vap L: 1998. Artifactually increased serum bicarbonate values in two horses and a calf with severe rhabdomyolysis. *Vet Clin Pathol* 27:85–90.

Constable PD: 2000. Clinical assessment of acid-base status: Comparison of the Henderson-Hallelbalch and strong ion approaches. *Vet Clin Pathol* 29:115–128.

Davies DR, Foster SF, Hopper BJ, et al: 2008. Hypokalaemic paresis, hypertension, alkalosis and adrenal-dependent hyperadrenocorticism in a dog. *Aust Vet J* 86:139–146.

Desmarchelie M, Lair S, Dunn M, et al: 2008. Primary hyperaldosteronism in a domestic ferret with an adrenocortical adenoma. *J Am Vet Med Assoc* 233: 1297–1301.

DiBartola S: 2006. Fluid Therapy in Small Animal Practice. 3rd ed. Saunders Elsevier, St. Louis.

Durocher LL, Hinchcliff KW, DiBartola SP, et al: 2008. Acid-base and hormonal abnormalities in dogs with naturally occurring diabetes mellitus. *J Am Vet Med Assoc* 232:1310–1320.

Flaminio MJ, Rush BR: 1998. Fluid and electrolyte balance in endurance horses. *Vet Clin North Am Equine Pract* 14:147–158.

Goff JP: 2000. Pathophysiology of calcium and phosphorus disorders. *Vet Clin North Am Equine Pract* 16: 319–337.

Grosenbaugh DA, Gadawski JE, Muir WW: 1998. Evaluation of a portable clinical analyzer in a veterinary hospital setting. *J Am Vet Med Assoc* 213:691–694.

Grove-White DH, Michell AR: 2001. Comparison of the measurement of total carbon dioxide and strong ion difference for the evaluation of metabolic acidosis in diarrhoeic calves. *Vet Rec* 148:365–370.

Heath SE, Peter AT, Janovitz EB, et al: 1995. Ependymoma of the neurohypophysis and hypernatremia in a horse. *J Am Vet Med Assoc* 207:738–741.

Hinchcliff KW, Reinhart GA, Burr JR, et al: 1997. Exercise-associated hyponatremia in Alaskan sled dogs: Urinary and hormonal responses. *J Appl Physiol* 83:824–829.

Hood VL, Tannen RL: 1998. Protection of acid-base balance by pH regulation of acid production. *N Engl J Med* 339:819–826.

Kang JH, Chang D, Lee YW, et al: 2007. Adipsic hypernatremia in a dog with antithyroid antibodies in cerebrospinal fluid and serum. *J Vet Med Sci* 69:751–754.

Looney AL, Ludders J, Erb HN, et al: 1998. Use of a handheld device for analysis of blood electrolyte concentrations and blood gas partial pressures in dogs and horses. *J Am Vet Med Assoc* 213:526–530.

Lorenz I: 2009. D-lactic acidosis in calves. *Vet J* 179:197–203.

Lorenz I, Lorch A: 2009. D-lactic acidosis in lambs. *Vet Rec* 164:174–175.

Matthews NS, Hartke S, Allen JC: 2003. An evaluation of pulse oximeters in dogs, cats and horses. *Vet Anaesth Analgesia* 30:3–14.

McCutcheon LJ, Geor RJ: 1996. Sweat fluid and ion losses in horses during training and competition in cool vs. hot ambient conditions: Implications for ion supplementation. *Equine Vet J Suppl* 54–62.

Free ebooks ==> www.Ebook777.com

Ohtsuka H, Ohki K, Tajima M, et al: 1997. Evaluation of blood acid-base balance after experimental administration of endotoxin in adult cow. *J Vet Med Sci* 59:483–485.

Ozaki J, Tanimoto N, Kuse H, et al: 2000. Comparison of arterial blood gases and acid-base balance in young and aged beagle dogs, with regard to postprandial alkaline tide. *J Toxicol Sci* 25:205–211.

Packer RA, Cohn LA, Wohlstadter DR, et al: 2005. D-lactic acidosis secondary to exocrine pancreatic insufficiency in a cat. *J Vet Intern Med* 19:106–110.

Paulson WD: 1996. Effect of acute pH change on serum anion gap. *J Am Soc Nephrol* 7:357–363.

Picandet V, Jeanneret S, Lavoie JP: 2007. Effects of syringe type and storage temperature on results of blood gas analysis in arterial blood of horses. *J Vet Intern Med* 21:476–481.

Rose RJ, Bloomberg MS: 1989. Responses to sprint exercise in the greyhound: effects on haematology, serum biochemistry and muscle metabolites. *Res Vet Sci* 47:212–218.

Roth L, Tyler RD: 1999. Evaluation of low sodium:potassium ratios in dogs. *J Vet Diagn Invest* 11:60–64.

Roussel AJ, Cohen ND, Holland PS, et al: 1998. Alterations in acid-base balance and serum electrolyte concentrations in cattle: 632 cases (1984–1994). *J Am Vet Med Assoc* 212:1769–1775.

Rubin SI, Toolan L, Halperin ML: 1998. Trimethoprim-induced exacerbation of hyperkalemia in a dog with hypoadrenocorticism. *J Vet Intern Med* 12:186–188.

Sattler N, Fecteau G, Girard C, et al: 1998. Description of 14 cases of bovine hypokalaemia syndrome. *Vet Rec* 143:503–507.

Schaafsma IA, van Emst MG, Kooistra HS, et al: 2002. Exercise-induced hyperkalemia in hypothyroid dogs. *Domest Anim Endocrinol* 22:113–125.

Schaer M, Halling KB, Collins KE, et al: 2001. Combined hyponatremia and hyperkalemia mimicking acute hypoadrenocorticism in three pregnant dogs. *J Am Vet Med Assoc* 218:897–899.

Taylor SM, Shmon CL, Adams VJ, et al: 2009. Evaluations of Labrador retrievers with exercise-induced collapse, including response to a standardized strenuous exercise protocol. *J Am Anim Hosp Assoc* 45:3–13.

Uystepruyst CH, Coghe J, Bureau F, et al: 2000. Evaluation of accuracy of pulse oximetry in newborn calves. *Vet J* 159:71–76.

Verwaerde P, Malet C, Lagente M, et al: 2002. The accuracy of the i-STAT portable analyser for measuring blood gases and pH in whole-blood samples from dogs. *Res Vet Sci* 73:71–75.

Wall RE: 2001. Respiratory acid-base disorders. *Vet Clin North Am Small Anim Pract* 31:1355–1367.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 6

PROTEINS, LIPIDS, AND CARBOHYDRATES

Ellen W. Evans, DVM, PhD

PLASMA PROTEINS

I. FUNCTIONS AND ORIGINS

A. Collectively, plasma proteins perform a nutritive function, exert colloidal osmotic pressure, participate in immune/inflammatory responses and the clotting process, and aid in the maintenance of acid-base balance. Individual proteins serve as enzymes, antibodies, coagulation factors, hormones, acute phase proteins, and transport substances. The major site of plasma protein synthesis is the liver, and the second major contributor is the immune system.

B. Fresh serum contains all of the plasma proteins except fibrinogen, factor V, and factor VIII. These are nonenzymatic coagulation proteins which are consumed during clot formation.

C. Age-related changes in plasma and serum protein concentrations occur in mammals and birds.

1. In mammals, plasma and serum protein concentrations are low at birth, increase after absorption of colostrum, decline over one to five weeks as colostrum is metabolized, and then increase to adult levels within six months to one year.

2. In adulthood, albumin decreases slightly over time while globulins, particularly immunoglobulins and acute phase proteins, progressively increase in old age. These changes over time should be considered when interpreting protein data.

3. In mammals, globulin and total serum protein concentrations tend to decrease during the last third of pregnancy. Just prior to parturition, globulin and total protein concentrations increase. Both albumin and total protein concentrations decrease during lactation.

4. Age-related changes in protein concentration also occur in birds, and significant differences exist between avian species. During egg production, albumin and total protein concentrations are decreased.

5. Age-related reference intervals are ideal for interpreting protein data but rarely are available.

D. Albumin represents 35% to 50% of the total serum protein concentration in domestic animals.

1. Albumin is synthesized by the liver and regulated by IL-1 and other cytokines.

2. A direct correlation exists between albumin turnover and body size. The half-life of albumin is eight days in the dog and 19 days in the horse.

3. Because of its abundance and small size, albumin accounts for 75% of the colloidal osmotic activity of plasma.

4. Many constituents of plasma are transported by albumin, and changes in albumin affect total circulating amounts of these constituents.

www.Ebook777.com

5. Albumin dampens platelet aggregation and augments antithrombin III; therefore, severely low plasma albumin may have pro-thrombotic implications.
6. Production of albumin decreases during the acute phase response.

E. Globulins are separated into α-, β-, and γ-globulins by electrophoresis (Figure 6.1).

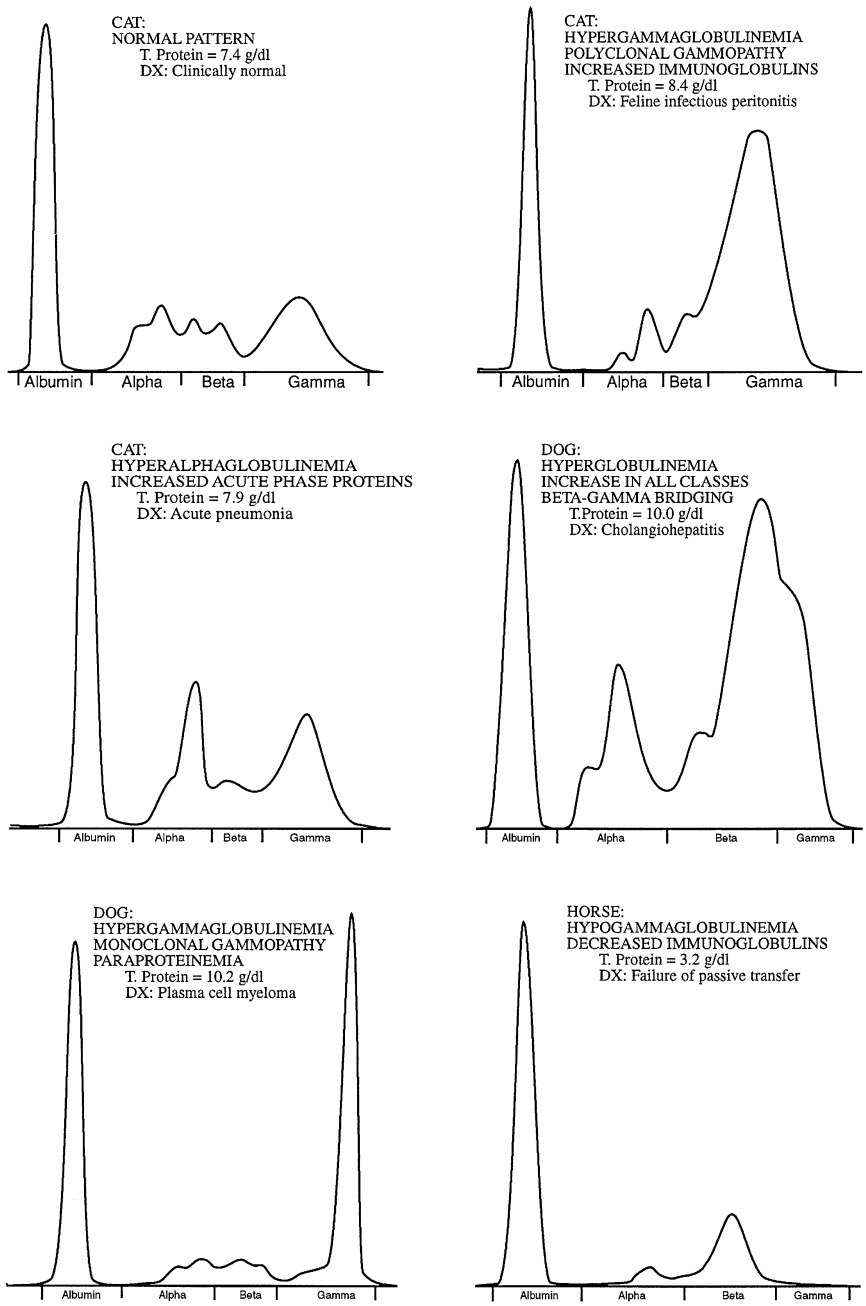


FIGURE 6.1. Examples of normal and abnormal serum electrophoretograms.

Free ebooks ==> www.Ebook777.com

1. α- and β-globulins
   a. Most of these globulins are synthesized by the liver.
   b. Lipoproteins and acute-phase proteins of inflammation are α- and β-globulins.
   c. Some immunoglobulins (IgM, IgA) may extend from the γ region into the β region of the serum protein electrophoretogram.
2. γ-globulins
   a. Most of the immunoglobulins migrate in the γ region of the serum protein electrophoretogram.
   b. Immunoglobulins are secreted by B lymphocytes and plasma cells in many tissues, particularly in the lymphoid organs.

II. METHODS OF MEASUREMENT

A. Total protein
1. "Total protein" usually is measured as serum protein.
2. The biuret method is a colorimetric, spectrophotometric technique that detects peptide bonds. It is highly specific for protein measurement
   a. The accuracy of this technique ranges from 1 to 10 g/dL and usually covers the range of protein concentrations encountered in plasma, serum, or body cavity fluids.
   b. The biuret method is inaccurate in determining protein concentrations that are less than 1 g/dL.
   c. Protein values for various species, particularly birds, vary according to protein standards that are used in the biuret method. Therefore, species-specific reference intervals should be established by the laboratory performing the assay.
3. The Lowry (Folin-Ciocalteau phenol) and modified Lowry (bicinchonic acid or BCA) methods are the preferred colorimetric methods for dilute fluids such as urine and cerebrospinal fluid (CSF).
   a. This assay detects tyrosine and tryptophan (amino acids) in small quantities (mg/dL) of protein.
4. Precipitation (trichloroacetic acid, sulfosalicylic acid, benzethonium chloride) and dye-binding (Coomassie Brilliant Blue RG 230) methods also have been used to quantify small amounts of protein found in urine and CSF.
   a. Precipitation methods are accurate for protein concentrations in the range of 2 to 200 mg/dL.
   b. The Coomassie blue-dye-binding method is accurate for protein concentrations in the range of 10 to 100 mg/dL. Although this technique underestimates globulins, this is not a serious handicap because albumin usually is the principal protein in CSF and urine.
   c. Different standards can be used to vary the albumin/globulin ratio and overcome the above problem.
5. Refractometry can be used to measure plasma, serum, or body cavity fluid protein.
   a. Changes in the refractive index of the specimen are proportional to the protein concentration.
   b. Temperature-compensated, hand-held refractometers are calibrated to read protein directly in g/dL. They are accurate for protein concentrations that are usually encountered in plasma, serum, and body cavity fluids.
   c. The assumption is made that other solutes in plasma and serum remain constant from sample to sample and are within the reference range.
      (1) Abnormally high concentrations of glucose, urea, sodium, or chloride may result in falsely high protein readings.
      (2) Lipemia alters light transmission through the specimen, producing an indistinct line of demarcation; falsely high protein readings usually result.
   d. The serum, plasma, or body cavity fluid must be clear for accurate determination of protein concentration
      (1) Hemolysis may cause a mild increase in protein concentration.
      (2) Turbidity from lipemia or cells may result in falsely high readings.
      (3) Icterus alters the color of the specimen but does not alter the reading.

www.Ebook777.com

e. Refractometry is not an accurate means of measuring total protein in birds. The biuret method is preferred. As a general guideline, the variability between avian species dictates that refractometric values should be validated with other methods of protein determination before using this technique in birds.

B. Albumin

1. The bromcresol green (BCG) dye-binding method is used by most veterinary laboratories to quantitate albumin.

a. Falsely high test results occur with low albumin concentrations because BCG binds to other proteins. Test results also may be inaccurate with very high albumin concentrations, which are seldom encountered clinically.

b. Anticonvulsants and certain antibiotics compete with albumin for BCG binding. A color shift in BCG dye, different from that caused by albumin, may result in a falsely low reading.

c. Variability in dye binding occurs between species.

(1) Canine and feline albumin binds BCG less avidly, giving falsely low values. However, feline albumin exhibits less variability in BCG binding than does canine albumin.

(2) Bovine and equine albumin binds BCG readily, causing falsely high values.

(3) Avian albumin has low affinity for BCG. Serum or plasma protein electrophoresis is the preferred method to quantitate albumin in birds, unless an avian standard is available for the BCG technique (most laboratory assays use bovine serum albumin as the standard).

(4) The use of species-specific standards may partially overcome some of these deficiencies in the BCG technique.

(5) Albumin concentration may be overestimated when heparinized plasma is analyzed instead of serum.

2. Bromcresol purple determination of albumin concentration is accurate in human specimens but is inaccurate in animal specimens.

3. Serum protein electrophoresis provides an alternative method to accurately quantitate albumin, if dye binding techniques prove to be unsatisfactory.

C. Globulin

1. Globulin concentrations in routine biochemical profiles usually are calculated by the following formula:

$$[\text{Total protein (g/dL)} - \text{albumin (g/dL)}] = \text{globulin (g/dL)}.$$

2. Globulins and albumin can be separated and directly quantified by serum electrophoresis (Figure 6.1).

a. Overlap of the various globulin fractions as well as species differences in normal patterns and numbers of peaks may obscure divisions between some protein fractions.

b. The midpoint of the electrophoretogram pattern usually lies between the α_2 and β_1 peaks.

c. Comparison of the patient's electrophoretogram to a normal electrophoretogram from the same species run on the same instrument often facilitates subsequent interpretation.

d. For mammals, the focal point of the electrophoretogram is the albumin peak, and fraction identification is based on migration distance in relation to albumin. For birds, considerable species variability exists in electrophoretograms, and it has been suggested that identification of the fibrinogen peak also should be taken into account in identifying protein fractions. Sample quality (lipemia, hemolysis) and storage conditions frequently alter results.

3. Immunochemical and radioimmunologic methods allow for specific identification and quantitation of individual globulins.

4. Several screening tests are available to detect failure of passive transfer of colostral antibody in foals and calves.

a. Serum IgG concentrations below 200 mg/dL at 24 to 48 hours after birth confirm failure of passive transfer, whereas values below 200 to 400 mg/dL suggest partial failure of passive transfer. Serum IgG greater than 800 mg/dL is required for adequate protection.

Free ebooks ==> www.Ebook777.com

b. Screening tests used to detect IgG above these minimal concentrations include the following:
(1) Zinc sulfate turbidity test
(2) Latex agglutination test
(3) Glutaraldehyde coagulation test
(4) Membrane filter and dipstick enzyme-linked immunoassay (ELISA) tests

5. Refractometric total serum protein values above 5 g/dL in neonates have been used to indicate colostral immunoglobulin absorption; however, false-negative findings limit the usefulness of this method.

D. The albumin/globulin (A/G) ratio is calculated mathematically. This calculated value has been used to aid in the interpretation of total protein values.

1. The A/G ratio will remain within the reference interval if both fractions are altered uniformly. Examples include the following:
a. Loss of both albumin and globulin via hemorrhage
b. Increased concentration of albumin and globulin from dehydration

2. The A/G ratio is abnormal if an alteration of one fraction predominates. Examples include the following:
a. Decreased A/G ratio with renal proteinuria and/or immunoglobulin production following antigenic stimulation.
b. Increased A/G ratio from lack of immunoglobulin production in adults or lack of colostrum absorption in foals and calves. The body does not overproduce albumin.

E. Fibrinogen

1. Fibrinogen concentration may be determined indirectly by refractometry using two centrifuged microhematocrit blood tubes.
a. The plasma protein concentration is measured using the first tube. The second microhematocrit tube is heated at 56°C for 10 minutes, and the fibrinogen is precipitated by heat denaturation. Following re-centrifugation, the plasma protein reading is determined again.
b. The mathematical difference between the two readings is assumed to represent the fibrinogen concentration (mg/dL).
c. This crude procedure may detect a fibrinogen concentration within the reference interval or the presence of hyperfibrinogenemia, but is too insensitive to detect hypofibrinogenemia.

2. Other methods are necessary to quantitate fibrinogen more accurately, especially to identify hypofibrinogenemia or normofibrinogenemia.
a. Determination of thrombin clotting time. The instrument then compares thrombin time (seconds to a standard curve) to determine fibrinogen concentration (mg/dL).
b. Direct measurement of fibrinogen by automated method (Chapter 4).

F. Acute phase protein methods include immunonephelometry, immunoblot, turbidometric immunoassays, latex agglutination, ELISA, radioimmunoassay, and in the case of haptoglobin, haptoglobin-hemoglobin binding assay and capillary electrophoresis. Automated spectrophotometric methods for commonly assayed acute phase proteins have been developed. Reagents and diagnostic kits for use on human samples are the most readily available, and many of the reagents cross-react across species. However, it is important to ensure that the particular assay being used is appropriate for the species of interest.

III. PROTEIN ABNORMALITIES (DYSPROTEINEMIAS)

A. Hyperproteinemia

1. Relative (dehydration) (Cases 6, 9, 14, 18, 22)
a. Water loss concentrates all plasma proteins proportionally; the A/G ratio is within the reference interval.

www.Ebook777.com

b. Dehydration is based on clinical findings only. Once the hydration status of the animal is known, day-to-day changes can be monitored by determining protein concentration (which is better than the hematocrit for this purpose). However, neither the protein concentration nor hematocrit is a reliable indicator if the magnitude of fluid loss is small (see discussion on dehydration in Chapter 5).

2. Hyperalbuminemia represents a relative increase in albumin concentration secondary to dehydration. Concurrent hyperglobulinemia also may be apparent. An absolute increase in albumin concentration is rare.

3. Hyperfibrinogenemia commonly occurs in inflammatory or neoplastic disease (Cases 6, 21) because fibrinogen is an acute-phase reactant.

a. Hyperfibrinogenemia is not detected with routine biochemical profiles because serum is usually evaluated instead of plasma.

b. Hyperfibrinogenemia is particularly useful as an indicator of early inflammation in cattle, sheep, and horses, and may precede the development of neutrophilia or other significant changes in the leukogram. It may also occur in the absence of changes in the leukogram, especially in cattle.

c. Dehydration causes relative increases in fibrinogen concentration that can be differentiated from true hyperfibrinogenemia by calculating the plasma protein/fibrinogen (PP/F) ratio (e.g., PP = 8.4 g/dL, F = 600 mg/dL, PP/F ratio = 8.4/0.6 = 14).

(1) PP/F ratio greater than 15 is consistent with dehydration or normofibrinogenemia (normal fibrinogen concentration).

(2) PP/F ratio below 10 is consistent with true hyperfibrinogenemia.

(3) Lack of precision in determining the fibrinogen concentration by the heat precipitation procedure can adversely affect the ratio, limiting its usefulness.

4. Hyperglobulinemia

a. Globulin concentration tends to increase with infection and inflammation and near term during pregnancy. In birds, globulins increase just prior to egg laying.

b. Acute-phase proteins cause mild increases in globulin concentration

(1) Most acute-phase proteins are α-globulins, but some are β-globulins (Figure 6.1).

(2) Hepatic synthesis of positive acute-phase proteins begins within 24 hours after acute tissue injury (e.g., inflammation, necrosis, surgery, infection, tumors, immune-mediated processes, etc.), and are also produced in response to estradiol, physical stress, or corticosteroid administration. Extra-hepatic production also occurs.

(3) Synthesis of acute-phase proteins is stimulated by IL-1β, IL-6, and TNF, which are released mainly from macrophages at the site of injury, but can also be released by other cells.

(4) Acute-phase proteins play a role in the immune response, provide protection against oxidative stress generated in the course of an inflammatory response, and have anti-infective properties. These functions usually enhance survival.

(5) Detection of acute-phase proteins may be useful in the early diagnosis of tissue injury and in monitoring the response to treatment and resolution of tissue trauma or inflammation. They are often more sensitive than the WBC count in identifying an inflammatory response. High values often correlate with severity of disease but do not necessarily predict outcome. Sustained high values of rapid-responding acute phase proteins, despite treatment, may suggest a poorer prognosis.

(6) While they are often increased in various tumors, they are more likely to be increased in lymphomas or tumors that are associated with significant inflammation. Acute-phase protein values do not consistently correlate with response to chemotherapy or relapse.

(7) Measurement of acute-phase proteins other than fibrinogen and albumin is not performed commonly in clinical practice, but is gaining more utility as assays become more readily available. In most cases, measurement of acute-phase proteins offers little advantage

Free ebooks ==> www.Ebook777.com

in screening for acute-phase response than does the presence of a decreased A/G ratio, but applications for measurement of specific positive acute phase proteins are growing.

(8) The acute-phase response, particularly which proteins predominate and the magnitude of their increase, varies between species and etiology. The patterns have not been thoroughly worked out for all species and inciting causes. Identification and monitoring of acute-phase responses are enhanced by assaying more than one acute phase protein and sampling multiple time points. The concentration of acute-phase proteins may increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) during inflammation or infection.

(9) Although acute-phase proteins are useful in screening for inflammatory processes and tumors, they are nonspecific as to mechanism and may increase during normal physiologic processes (e.g., pregnancy, perinatal period, parturition, uncomplicated surgery) and in hepatic lipidosis in cattle.

(10) Increases in some acute-phase proteins (haptoglobin, ceruloplasmin, fibrinogen, and others) have been proposed as means of pregnancy diagnosis in dogs, but care must be taken to rule out confounding inflammatory conditions and collect samples at precise intervals after the mating dates.

(11) Rapid-reacting acute-phase proteins increase rapidly (within 24 hours) and have relatively short half-lives. These include serum amyloid A- and C-reactive protein. The second group rises later in the inflammatory response (within 48 hours or later) and may be increased for up to two weeks. Examples of late responders are haptoglobin, fibrinogen, and LPS-binding protein. The measurement of at least one rapid responder and one later responder increases the ability to detect acute-phase responses and monitor progress.

(12) Calculation of acute phase index (API) increases sensitivity and has been used in research settings, particularly in evaluating herd health. The calculation is based on multiplying the value for a rapid-reacting positive acute-phase protein with that of a late-responding positive acute-phase protein and dividing the product by one or the product of two (one rapid and one late) negative acute-phase protein. The acute-phase proteins used should be appropriate for the species in question.

(13) Acute-phase proteins can be measured at local sites (cerebrospinal fluid, peritoneal fluid, joint fluid, etc.) to aid in the diagnosis of inflammatory conditions.

(14) Serum amyloid A shows the greatest cross-species utility. In addition, for dogs, C-reactive protein, and for dogs and cats, α_1-acid glycoprotein are also particularly useful; and haptoglobin is a sensitive indicator in ruminants and horses. Major acute phase protein, haptoglobin, and C-reactive protein generally provide strong responses in pigs, and α_1-acid glycoprotein has shown utility in assessing avian acute-phase responses.

(15) Saliva can be used as a surrogate for blood in assessing acute-phase proteins.

(16) The major positive acute-phase proteins include the following:

(a) Fibrinogen has been used as an index of inflammation in many animal species, particularly in horses and cattle.

(b) Certain coagulation proteins (factor V, factor VIII, and fibrinogen). Increases in these nonenzymatic factors may accelerate hemostasis (Chapter 4).

(c) C-reactive protein (CRP; a major responder in dogs, horses, and pigs). Response is evident within four hours, peaks after 24 hours, and returns to baseline soon after removal of insult. CRP may be a useful parameter for monitoring recovery from acute pancreatitis.

(d) Serum amyloid A (SAA; useful in birds, cattle, cats, dogs, pigs, sheep, goats, and horses); also increases in reactive or hereditary systemic amyloidosis. SAA is considered more useful than fibrinogen in that the increase in SAA occurs sooner and is of greater magnitude than that of fibrinogen. For cats with pancreatitis, monitoring SAA may be a more sensitive means of assessing response to treatment than monitoring feline trypsin-like immunoreactivity (fTLI).

www.Ebook777.com

(e) Haptoglobin (Hp; useful in cows, pigs, horses, cats, dogs, sheep, and goats). Considered more sensitive than fibrinogen. This protein binds to free hemoglobin and is decreased in intravascular hemolysis (Chapter 1). Increases occur from corticosteroid administration regardless of underlying disease, as well as naturally occurring hyperadrenocorticism. Glycosylation varies in various diseases, which has potential utility for differential diagnosis.

(f) Ceruloplasmin, a copper transport protein (Cp; cattle, horses, birds, and in pregnancy diagnosis in the dog)

(g) Complement components, especially C3

(h) α_1-acid glycoprotein (AGP), also called acid-soluble glycoprotein (chickens, cattle, goats, sheep, dogs, and cats). AGP also can be produced by lymphocytes, and high serum levels are often seen in lymphoma. High serum levels have been demonstrated in neoplasms of other origins, as well. Increases also have been shown to have particular utility in the diagnosis of feline infectious peritonitis (FIP) and can be measured in both serum and peritoneal fluid. The degree and nature of glycosylation of AGP shows promise in differential diagnosis of disease states and specific infections, particularly active FIP infection.

(i) α_1-antitrypsin, a protease inhibitor: the literature is conflicting regarding its usefulness in domestic species.

(j) α_2-macroglobulin appears to be of limited use in domestic species other than rodents.

(k) Major acute phase protein; also called "porcine major acute protein" (MAP; pigs)

(l) TNF-α (cats)

(m) Ovotransferrin (birds)

(n) LPS-binding protein (LBP; identification of infection in cattle)

(o) Hepcidin

(p) Sialic acid, while not an acute phase protein, has been measured as an indicator of the acute phase response. Increases are considered secondary to increased sialylation of α_1-acid glycoprotein or shedding from damaged cell membranes.

(17) Major negative acute-phase proteins include the following:

(a) Albumin

(b) Transferrin, the iron transport protein (Tf; cattle, pigs)

(c) Transthyretin (TTR; prealbumin)

(d) α_2-macroglobulin (cattle)

(e) Retinal binding protein

(f) Cortisol binding protein

(g) α-1 apolipoprotein (apoA1)

c. In nephrotic syndrome, increases in low density lipoproteins (LDL, β-lipoprotein), VLDL (pre-β-lipoprotein), and α_2-macroglobulin result in an increase in α_2-globulins on the serum electrophoretogram.

d. Transferrin, IgM, hemopexin, and/or complement (C3) may increase in acute liver disease, resulting in increases in β-globulins on the serum electrophoretogram.

e. Acute-phase protein, especially haptoglobin measurement, can be used to assess herd health status and stress levels in pigs at various husbandry stages.

f. Healthy Greyhounds have low α-globulin peaks on the serum electrophoretogram compared with other breeds; this may be due to lower concentrations of haptoglobin and α_1-acid glycoprotein.

g. Immunoglobulins (chronic phase proteins) can cause marked increase in the globulin fractions on the serum electrophoretogram.

(1) Immunoglobulins are located in the β and γ regions of the serum electrophoretogram (Figure 6.1).

Free ebooks ==> www.Ebook777.com

(2) Selective increased concentrations of β-globulins seldom occur; they usually are associated with increased concentration of other globulins.

(3) Bridging of β and α regions in the serum electrophoretogram is highly suggestive of chronic active hepatitis.

(4) Polyclonal gammopathies are characterized by an increased globulin fraction with a broad-based electrophoretic peak comprised of a heterogeneous mixture of immunoglobulins Figure 6.1).

(a) The protein increase usually involves the γ-globulin region but may extend into the β region, especially during periods when an IgM response is prominent.

(b) Polyclonal gammopathy is associated with chronic antigenic stimulation occurring in inflammatory diseases, immune-mediated diseases, and liver disease (Cases 7, 8, 9, 11).

(5) Monoclonal gammopathy (paraproteinemia) is characterized by an increased globulin fraction with a narrow-based electrophoretic peak that is no wider than the albumin peak (Figure 6.1).

(a) The monoclonal peak or spike is caused by a homogeneous immunoglobulin molecule that is produced by a single clone of B lymphocytes or plasma cells.

(b) Monoclonal peaks may be located in γ, β-, or α-globulin regions.

(c) Monoclonal gammopathy occurs most commonly with lymphoid neoplasia including plasma cell myeloma, lymphoma, chronic lymphocytic leukemia, and macroglobulinemia. Infrequently, monoclonal gammopathy may be observed with non-neoplastic disorders such as canine amyloidosis, canine ehrlichiosis, canine visceral leishmaniasis, feline infectious peritonitis, and plasmacytic gastroenterocolitis. An idiopathic, benign monoclonal gammopathy also occurs but is rare.

(d) Monoclonal gammopathy may be accompanied by overproduction of immunoglobulin light chains. Light chains (Bence-Jones proteins) are rapidly filtered from plasma into urine by the kidneys. Bence-Jones proteins are best detected by electrophoresis of concentrated urine specimens (Chapter 9).

h. The A/G ratio is decreased in hyperglobulinemia because of marked production of globulin, especially immunoglobulin. The albumin concentration remains within the reference interval or is decreased slightly (possibly by a compensatory mechanism to maintain plasma oncotic pressure).

B. Hypoproteinemia

1. Relative hypoproteinemia can occur with dilution of plasma protein by excess fluid. This type of hypoproteinemia is observed with overzealous administration of intravenous fluids, interstitial water shifts into the plasma after acute blood or plasma loss, and, occasionally, pregnancy.

2. Hypoalbuminemia

a. Diminished production of albumin is associated with pregnancy, lactation, intestinal malabsorption, malnutrition, cachexia secondary to neoplasia, exocrine pancreatic insufficiency, and chronic liver disease. Egg production is another cause of hypoalbuminemia in birds. Hypoalbuminemia is not a consistent finding in horses with severe liver disease; the presence of a postalbumin shoulder on the serum electrophoretogram is highly suggestive of liver disease in this species.

b. Accelerated loss of albumin occurs with hemorrhage, proteinuria of renal disease, protein-losing enteropathy, severe exudative skin disease, burns, intestinal parasitism, and high-protein effusions.

c. Nephrotic syndrome is recognized by the concomitant findings of hypoalbuminemia, azotemia, and hypercholesterolemia.

d. Albumin is mildly decreased in acute tissue injury or inflammation; it is a negative acute phase reactant. This mild decrease in albumin is offset by an increase in positive acute-phase reactants found in the α- and β-globulin fractions, and hypoproteinemia usually does not occur.

www.Ebook777.com

e. If selective loss (e.g., glomerular disease) or deficient production (e.g., hepatic insufficiency) of albumin occurs, the A/G ratio will be decreased (Cases 13, 19). If there is a concomitant loss of or failure to synthesize globulins (e.g., hemorrhage, exudation, protein-losing enteropathies, malassimilation), then panhypoproteinemia will occur and the A/G ratio will remain within the reference interval (Cases 1, 16, 25).

3. Hypoglobulinemia

a. Failure of passive colostral transfer or colostral deprivation in neonates leads to a very low γ-globulin concentration, because animals have very low immunoglobulin levels at birth (Figure 6.1).

b. Severe combined immunodeficiency disease (SCID) of Arabian foals and other animals results from failure to produce B lymphocytes (that synthesize immunoglobulins) and T lymphocytes (that function in cell-mediated immunity). SCID is characterized by low concentrations of endogenous immunoglobulin after catabolism of maternal colostral antibodies and a concurrent profound lymphopenia in the blood.

c. Other diseases characterized by a deficiency of immunoglobulin include agammaglobulinemia; selective IgM, IgA, and IgG deficiencies; and transient hypogammaglobulinemia.

d. With the above conditions, globulins are selectively reduced, and a high A/G ratio occurs. Hypoglobulinemia is more likely to be observed with IgG deficiency because it is present in the largest quantity in serum. Because IgA and IgM concentrations are generally very low, their absence seldom causes hypoglobulinemia.

e. Globulins may be lost concurrently with albumin during hemorrhage, exudation, and protein-losing enteropathy. In addition, deficient protein synthesis in severe malnutrition, maldigestion, and malabsorption may be associated with hypoglobulinemia as well as hypoalbuminemia. The A/G ratio usually remains within the reference interval.

4. Hypofibrinogenemia is usually caused by disseminated intravascular coagulation (Case 11). Routine serum protein tests, including the heat precipitation test, are too insensitive to detect hypofibrinogenemia in the plasma. The thrombin time test is the preferred method to detect hypofibrinogenemia.

IV. SERUM OR PLASMA COLLOID OSMOTIC PRESSURE (COP)

A. Physiologic considerations

1. Serum or plasma COP is the contribution to effective osmolality from macromolecules. It is the principal force opposing the exit of fluid from the vascular system.

2. Decreased COP produces edema, ascites or hydrothorax, and increased total body water.

3. In health, plasma proteins are responsible for COP, with albumin being the major contributor. Globulins contribute approximately 35% (human data).

4. Synthetic colloids, given to animals to increase COP, produce approximately eight times greater COP per unit weight than plasma proteins.

B. Determination of COP

1. Colloid osmometry

a. Colloid osmometers give the most accurate evaluation of COP, especially in animals that have been treated with synthetic colloids.

b. Colloid osmometers have two chambers separated by a semi-permeable membrane. Serum or plasma is placed on one side of the membrane and saline is placed on the other side.

c. Decrease in pressure on the saline side is caused by the flow of water to the sample side of the chamber. Changes in pressure are reported in mm Hg.

d. COP for healthy animals varies by species, with feline greater than canine, greater than equine, greater than bovine COP. Published values range from 20 to 30 mm Hg.

e. Individual laboratories should establish reference intervals for their analytical instrument.

Free ebooks ==> www.Ebook777.com

f. Colloid osmometry is the only method of evaluating COP from a patient that has been treated with synthetic colloids.

2. Estimation of COP from serum or plasma protein concentration

a. Protein concentration can be used to estimate COP, but does not give accurate values.

b. The relationship between protein concentration and COP is non-linear. Multiple quadratic equations have been developed to estimate COP in domestic animals from protein and albumin concentrations and A/G ratios.

c. These complicated calculations have limited usefulness due to variations in COP from changes in the protein composition of plasma of sick animals and from the therapeutic use of synthetic colloids.

V. TROPONINS AND OTHER INDICATORS OF CARDIAC DISEASE

A. Troponins are intracellular myofibrillar proteins involved in calcium-ion regulated striated muscle contraction.

1. There are several isoforms.

2. The major isoforms useful in assessing cardiac muscle integrity are cardiac troponins I and T (cTnI and cTnT).

a. There is considerable cross-species homology for both cTnI and cTnT.

b. In human medicine, mutations in troponins are associated with dilated and hypertrophic cardiomyopathies, and mutations in the feline cTnI gene may have a role in feline familial hypertrophic cardiomyopathy.

c. cTnI and cTnT are released into blood during myocardial damage.

d. cTnI appears to be more sensitive than cTnT in assessing myocardial damage; however, cTnT increases have been associated with more severe injury.

e. The magnitude of increase for cTnI generally correlates with severity of injury.

(1) Higher cTnI values correlate with higher thyroxine values in feline hyperthyroidism.

(2) The magnitude of cTnI increases in horses involved in endurance rides correlates with the duration of the ride.

(3) cTnI values have correlated with severity of cardiac involvement and are negatively correlated with outcome in gastric dilatation-volvulus in dogs.

f. cTnI has shown utility in the differential diagnosis of cardiac vs. noncardiac causes of dyspnea in cats.

g. Greyhounds normally have higher cTnI in the absence of cardiac disease than do other breeds.

B. Markers of neurologic responses to heart failure such as natriuretic peptides or vascular involvement such as endothelin also have been used to identify cardiac disease or determine severity, but the assays are less readily available and generally have little advantage over troponins.

1. Natruiuretic peptides and indicators of sympathetic nervous system cardiovascular responses are early responders in cardiac injury, followed by endothelin, which indicates alterations in the vasculature.

2. In dogs, beta-type natriuretic peptide has shown greater utility in the differential diagnosis of cardiac vs. non-cardiac causes of dyspnea than has cTnI or atrial natriuretic peptide.

PLASMA LIPIDS

I. TYPES AND ORIGINS

A. There are five major types of lipid in plasma.

1. Cholesterol

2. Cholesterol esters

www.Ebook777.com

3. Triglycerides (triacylglycerols)
4. Phospholipids
5. Nonesterified fatty acids (long-chain fatty acids)

B. Exogenous lipids

1. Most of the dietary lipid consists of long-chain triglycerides with a smaller proportion of cholesterol, cholesterol esters, phospholipids, and medium-chain triglycerides.
2. Lipid is digested into monoglycerides and free fatty acids by pancreatic lipase; digestion is facilitated by emulsification of lipids by bile acids. Following digestion, micelles are formed from monoglycerides, free fatty acids, cholesterol, bile salts, and fat-soluble vitamins. These micelles are absorbed by jejunal enterocytes. The remaining bile salts and medium-chain fatty acids may be absorbed directly into the portal blood.
3. Jejunal enterocytes degrade the micelles into fatty acids, monoglycerides, and cholesterol. Subsequently, chylomicra are synthesized from triglycerides (formed from glycerol and fatty acids), cholesterol esters, cholesterol, phospholipid, and apolipoproteins (peptides involved in lipid transport). The chylomicra are secreted into intestinal lymph of the lacteals.
4. Chylomicra enter plasma via the thoracic duct. Following hydrolysis by plasma lipoprotein lipase, the fatty acids and glycerol are absorbed, especially by adipocytes and hepatocytes, where they are stored as triglycerides. Alternatively, the hepatocytes can oxidize certain lipids for energy.

C. Endogenous lipids (Figure 6.2)

1. Most lipids are transported in plasma attached to apolipoproteins. These lipid-peptide complexes are very large and consist of varying quantities of triglyceride, cholesterol, cholesterol esters, and phospholipid. They are called lipoproteins.
2. Lipoproteins are synthesized by the liver and small intestine and secreted into plasma.
3. The fate of endogenous lipids is complex and not well characterized in most species. Plasma lipoprotein lipase and the presence of appropriate cell receptors are required for clearance of lipids from blood.

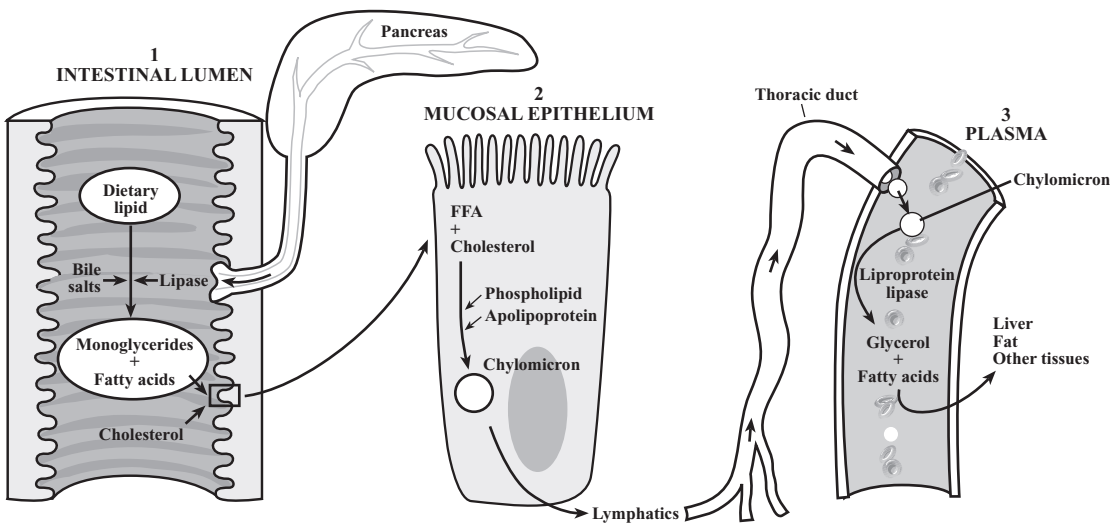


FIGURE 6.2. Overview of lipid metabolism. Panel 1: Ingested dietary lipids are digested by pancreatic lipase and emulsified by bile salts to monoglycerides and fatty acids. Micelles are formed (from monoglycerides, fatty acids, and cholesterol), and absorbed by enterocytes. Panel 2: Micelles are degraded in the enterocyte and chylomicra are formed from free fatty acid, cholesterol, phospholipid, and apolipoprotein. The chylomicra are secreted into the lymphatics. Panel 3: Chylomicra enter the blood plasma via the thoracic duct. They subsequently are metabolized by hepatocytes, adipocytes, and other cells.

Free ebooks ==> www.Ebook777.com

4. Exercise, diet, body condition, and reproductive cycle affect plasma lipid profiles.
5. Plasma lipoproteins are characterized by their density via ultracentrifugation and electrophoretic mobility. Lipoprotein density depends upon the ratio of protein to lipid. Lipoproteins with the highest density have the greatest proportion of protein. The main lipoprotein fractions or classes, listed in order of increasing density, include the following:
   a. Chylomicra. Chylomicra are synthesized by enterocytes and are the least dense of the lipoproteins. They are comprised primarily of triglycerides of dietary origin, which they transport in the blood.
   b. Very-low-density lipoproteins (VLDL). The lipid component of VLDLs consists of triglyceride, cholesterol, and phospholipid in an approximate ratio of 4:1:1. They are synthesized primarily in the liver. VLDLs export hepatic triglyceride and cholesterol and distribute triglyceride to adipose tissue and striated muscle.
   c. Intermediate-density lipoproteins (IDL). IDLs are formed in the vasculature following lipoprotein hydrolysis of VLDLs. IDLs contain approximately equal quantities of cholesterol and triglyceride in addition to lipoprotein. They subsequently are catabolized to LDLs (below). Because of their transient nature, they frequently are not quantitated.
   d. Low-density lipoproteins (LDL). LDLs are rich in protein with small amounts of cholesterol and triglyceride. They are formed in the vasculature when hepatic lipoprotein lipase removes additional lipid from IDLs. LDLs distribute cholesterol to peripheral tissues.
   e. High-density lipoproteins (HDL). HDLs contain cholesterol, protein, and phospholipid with very little triglyceride (hence their "high" density). They are formed in the intestine and liver. There are three types of HDL, designated HDL_1, HDL_2, and HDL_3. HDL_1 has more cholesterol and less protein than HDL_2. HDLs transport cholesterol to the liver.
6. There are large species differences in lipoprotein profiles and the percentage of total cholesterol carried by each lipoprotein class. Although reports of percent and absolute values of VLDL, HDL, and LDL cholesterol vary, several generalizations can be made.
   a. In humans, the majority of cholesterol is transported as LDL cholesterol.
   b. In pigs, more than half of the total cholesterol circulates as LDL and VLDL combined. The lipoprotein profile of pigs resembles that of humans.
   c. In ruminants and horses, the majority of cholesterol circulates as HDL.
   d. Cats, dogs, and birds (males and non-egg-laying females) have the highest percentage of HDL of the domestic species characterized to date. Dogs have HDLs 1 and 3, while cats have HDLs 2 and 3. Egg-laying female birds have decreased HDL and high levels of VLDL compared with their non-egg-laying counterparts.
7. Dogs and cats generally are resistant to atherosclerosis, even in disease states associated with hyperlipidemia. This is due in part to the very low concentration of LDLs in these species. In addition, cats and dogs lack cholesterol ester-transfer protein (CETP), which is responsible for the exchange of cholesterol and triglycerides between lipoproteins. A lack of CETP is associated with more cholesterol transported as HDL. Although dogs and cats are resistant to atherosclerosis, instances of lipid-related vascular disease have been reported with primary and secondary hyperlipidemic disorders in dogs and cats.
8. Dogs have significant interbreed differences in lipoprotein profiles but do not have significant differences in total plasma cholesterol and triglyceride concentrations.
9. Nonesterified fatty acids (NEFAs, long-chain fatty acids, LCFAs)
   a. NEFAs are synthesized in liver, adipose, and mammary tissue. Synthesis is regulated by glucagon (decreases synthesis) and insulin (promotes synthesis). The liver of ruminants makes only a small contribution to NEFA concentration in health.
   b. NEFA synthesis increases with high-carbohydrate/low-fat diets.
   c. NEFA synthesis decreases with fasting, diabetes mellitus, and high-fat/low-carbohydrate diets.
   d. During states of negative energy balance and increased lipolysis, NEFAs are released into plasma, processed in the liver, and can become triglycerides or acetyl-CoA. As plasma NEFAs

www.Ebook777.com

increase, much of the acetyl-CoA undergoes combustion in the tricarboxylic acid (TCA) cycle for energy or is converted to ketones.

e. Examples of conditions associated with increased plasma NEFAs are hyperlipidemias with increased lipase activity; diabetes mellitus; equine fasting hyperlipidemia; postexercise ketosis in horses and dogs; hepatic lipidosis in cats, horses, and cattle; equine hyperadrenocorticism; and bovine ketosis.

f. NEFAs may be measured in ruminants to assess metabolic status in conditions of altered lipid or carbohydrate metabolism, but are not routinely evaluated in other species.

10. Ketones are organic anions produced by fatty acid breakdown. In health, they do not appear in significant quantities in plasma or urine. Because of the complex interrelationship between lipid and carbohydrate metabolism, ketones are discussed in the carbohydrate section of this chapter.

II. MEASUREMENT AND CLINICAL SIGNIFICANCE OF PLASMA LIPIDS

A. Lipemia-refrigeration test. If a plasma or serum specimen is refrigerated for four to eight hours, turbidity indicates the presence of triglyceride-rich lipoproteins and/or chylomicra.

1. A flocculent layer on top with clearing below indicates chylomicra.

2. Persistent turbidity suggests triglyceride-rich lipoproteins (e.g., VLDL).

3. If a cream layer is seen on top with turbidity below, both chylomicrons and triglycerides are increased.

B. Serum cholesterol determination

1. Increased serum cholesterol concentrations reflect increased concentration of the cholesterol-rich lipoproteins (e.g., LDL, HDL). These lipoprotein increases commonly occur secondary to endocrine, hepatic, or renal diseases. Inherited or primary disorders of cholesterol metabolism are rare in domestic animals with the exception of Miniature Schnauzer dogs.

2. Serum cholesterol concentration generally is inversely related to thyroid hormone activity in dogs.

C. Serum triglyceride determination

1. Fasting samples should be analyzed because postprandial hypertriglyceridemia occurs four to six hours after eating, regardless of diet type. High-fat diets may cause postprandial lipemia composed of triglyceride-rich chylomicra.

2. Hypertriglyceridemia usually indicates chylomicronemia and/or increased VLDL. These changes may be postprandial if the patient was not fasted or may be secondary to endocrine, hepatic, pancreatic, or renal disease.

3. Lipoprotein lipase clears triglyceride-rich lipoproteins from the plasma. The activity of this enzyme is enhanced by insulin, thyroid hormone, and heparin (the heparin effect actually may be due to contamination of heparin products with lipoprotein lipase activator). Lipoprotein lipase activity increases in certain conditions such as equine hyperlipemia.

D. Lipoprotein determination

1. Combined ultracentrifugation and precipitation techniques have been used, and assays are also available for direct measurement of HDL and LDL. Formulas that calculate VLDL on the basis of HDL and total cholesterol concentrations are based on human VLDL triglyceride content. These formulas should not be used to calculate VLDL concentration in specimens from animals.

2. Serum lipoproteins also can be identified and quantified by their electrophoretic position in relation to those of serum proteins.

3. In general, HDLs migrate in the α region and LDLs in the β region of the lipoprotein electrophoretogram; however, considerable species variation exists in lipoprotein electrophoretic patterns. Five zones are present in canine lipoprotein electrophoretic patterns. The α_1-globulin zone contains HDL_2, the α_2-globulin zone contains HDL_1, the pre-β-globulin zone contains VLDL, the

Free ebooks ==> www.Ebook777.com

β-globulin zone contains LDL, and chylomicra remain at the origin of the electrophoretogram. In bovine lipoprotein electrophoretograms, LDLs may migrate in the α or β region.

4. Because routine serum protein electrophoresis only stains for the protein component (apolipoprotein) and not for the lipid component of lipoproteins, this procedure is not specific enough to allow adequate assessment of the lipoprotein profile.

5. Techniques to evaluate human lipoproteins appear suitable for specimens from cats and horses, but not all are suitable for specimens from dogs.

6. Most commercial laboratories analyze and interpret lipoprotein data on the basis of human patterns. Interpretation of veterinary data should be done by individuals who are familiar with animal lipoprotein electrophoretograms.

E. Nonesterified fatty acid determination

1. NEFAs are quantified by colorimetric spectrophotometry. They are extracted from serum or plasma with organic solvents and are chemically converted to cobalt or copper salts. The color intensity of the reaction is proportional to NEFA concentration.

2. NEFAs also may be measured by enzymatic assays.

3. Plasma lipase may degrade NEFAs in improperly handled specimens. Degradation of NEFAs by plasma lipase can be prevented.

a. Specimens should be analyzed immediately.

b. If a variable delay is anticipated prior to analysis of the specimen, paraoxon can be added to inhibit plasma lipase activity. Alternatively, after the blood specimen is centrifuged immediately, the plasma is removed and frozen for future analysis.

F. Lipoprotein lipase determination. Samples are collected before and 15 minutes after an intravenous injection of heparin (90 IU/kg) in dogs or before and 10 minutes after an intravenous injection of heparin (45 IU/kg) in cats. Cholesterol and triglyceride concentrations are measured, and the heparin-mediated lipoprotein lipase release should result in decreased triglycerides and cholesterol. Failure to decrease these values suggests decreased or absent lipoprotein lipase activity.

G. Adipokine assays. Currently available assays are immunology-based (RIA or ELISA). Although considerable cross-species homology exists, it is important to ensure that the assay used is appropriate for the species of interest.

III. HYPERLIPIDEMIA (TABLE 6.1)

A. Hyperlipidemia is an increase in plasma lipids (e.g., triglyceride, cholesterol, phospholipid). Increased nonesterified fatty acids alone do not constitute hyperlipidemia

1. In clinical practice, the term hyperlipidemia usually refers to either hypertriglyceridemia or hypercholesterolemia or both.

2. Postprandial hyperlipidemia is a transient rise in blood lipids that follows ingestion of a fatty meal. This form of hyperlipidemia is primarily due to the presence of chylomicrons.

3. Fasting or persistent hyperlipidemia is the presence of excess lipids in the blood following at least a 12-hour fast before the blood specimen was obtained. As the name implies, lipemia is a persistent rather than a transient phenomenon.

B. Hyperlipoproteinemia is used synonymously with hyperlipidemia because both triglyceride and cholesterol in plasma are contained in special complexes called lipoproteins.

C. Primary hyperlipidemia encompasses proven or suspected hereditary alterations in lipoprotein metabolism. These conditions generally are infrequent to rare, but have been reported in dogs and cats.

1. Idiopathic hyperlipidemia with hypertriglyceridemia, hypercholesterolemia, and hyperchylomicronemia. This condition occurs in Miniature Schnauzers, is probably hereditary, and

www.Ebook777.com

TABLE 6.1.
CAUSES OF HYPERLIPIDEMIA.

Primary hyperlipidemia (infrequent to rare)
- Congenital lipoprotein lipase deficiency (mongrel puppy)*
- Familial hyperlipoproteinemia (Beagles)
- Hypercholesterolemia with corneal lipodystrophy (dogs)
- Hypertriglyceridemia (Brittany Spaniels)*
- Idiopathic hyperlipoproteinemia of Miniature Schnauzers*
- Inherited hyperchylomicronemia (primary hypercholesterolemia) of cats*
- Hypertriglyceridemia in Burmese cats*

Secondary hyperlipidemia (relatively common)
- Acute pancreatitis*
- Cholestasis
- Diabetes mellitus*
- Diets high in saturated fat or cholesterol
- Enterocolitis (horses)
- Equine hepatic lipidosis syndrome (ponies, mules, horses)*
- Hepatic disease (lipidosis)*
- Hyperadrenocorticism, exogenous corticosteroids*
- Hypothyroidism*
- Nephrotic syndrome
- Postprandial*

*Lipemia may occur in these conditions.

is associated with excess VLDLs. Similar syndromes have been reported in Beagles, Brittany Spaniels, mixed-breed dogs, and cats.

2. Insulin-dependent hypertriglyceridemia. This condition has been reported in dogs.

3. Inherited lipoprotein lipase deficiency or familial hyperchylomicronemia of cats. This disease results in increased plasma concentrations of cholesterol and triglycerides. Affected individuals may have xanthomas, granulomas, lipemia retinalis, and peripheral neuropathies. Familial lipoprotein lipase deficiency also has been reported in dogs.

4. Primary hypercholesterolemia. This condition has been observed in dogs with corneal lipodystrophy.

5. Primary hypercholesterolemia of Briard dogs. Affected Briards have an increased HDL cholesterol concentration but appear clinically healthy.

6. Lipid aqueous in Burmese cats. Affected cats have moderately increased triglycerides (VLDL and chylomicrons). Lipoproteins cross the blood-aqueous barrier, resulting in "blue eye."

D. Conditions causing secondary hyperlipidemia are relatively common and include the following:

1. Postprandial hyperlipemia. Following ingestion of a meal, particularly one high in fat, hypertriglyceridemia occurs due to chylomicronemia. Dietary fat supplementation of horses may diminish postprandial plasma hypertriglyceridemia due to increased lipoprotein lipase activity.

2. Hypothyroidism (Case 28). In hypothyroidism, cholesterol use decreases while cholesterol synthesis increases. LDL cell receptor expression and lipoprotein lipase activity are reduced. Hyperlipidemia is variable, ranging from mildly increased cholesterol and HDL concentrations to marked lipemia with hypercholesterolemia (above 1,000 mg/dL), hypertriglyceridemia, and panhyperlipoproteinemia.

Free ebooks ==> www.Ebook777.com

3. Diabetes mellitus. In diabetes mellitus, lack of insulin results in reduced lipoprotein lipase activity (insulin enhances lipoprotein lipase activity). Lipolysis is increased as lipids are used as an alternative source of energy. Marked hypertriglyceridemia is associated with increased VLDL concentration; cholesterol concentration is mildly increased. Hyperchylomicronemia also may be present.

4. Equine hepatic lipidosis syndrome of pony mares and horses. This syndrome is associated with decreased feed consumption and a negative nitrogen balance in pregnant, lactating, recently transported, or debilitated animals. Hyperlipidemia varies from mild hypertriglyceridemia with clear plasma to marked lipemia with severe hypertriglyceridemia (as high as 10,000 mg/dL), increased NEFAs, and increased VLDLs. Plasma ketones also may be increased. Affected horses may be azotemic.

5. Other causes of secondary hyperlipidemia exist in which the mechanism and characteristics remain unclear.

a. Acute necrotizing pancreatitis. This condition is observed in dogs. The necrotic pancreas is thought to release inhibitors of lipoprotein lipase. Both triglyceride and cholesterol concentrations are increased. Hyperlipidemia also may increase the risk of pancreatitis.

b. Hepatic disease. Some dogs with cholestasis may have hypercholesterolemia and hypertriglyceridemia, but the hyperlipidemia usually is not of sufficient magnitude to be diagnostically useful. Feline hepatic lipidosis syndrome is not associated with lipemia, but increases in triglycerides and enrichment of LDL with triglycerides and of HDL with cholesterol have been reported. Cholestasis and icterus may be prominent.

c. Nephrotic syndrome. In nephrotic syndrome, hypercholesterolemia is secondary to enhanced synthesis of cholesterol-containing lipoproteins. Lipoprotein synthesis presumably is stimulated by hypoalbuminemia and decreased plasma oncotic pressure (Case 19). Sometimes triglyceride concentration also is increased.

d. Exogenous corticosteroid administration. Excess administration of corticosteroids may result in hypertriglyceridemia from increased lipolysis, insulin resistance, and decreased lipoprotein lipase activity. Moderate hypercholesterolemia, predominantly due to increased LDLs, may occur. Decreased liver LDL receptor activity has been suggested as the mechanism for these changes. Hyperlipidemia in uncomplicated, naturally occurring canine hyperadrenocorticism is mild, if present. Although increased concentrations of NEFAs and ketones often accompany equine hyperadrenocorticism, hyperlipidemia/lipemia is not a common feature of this disease.

e. Hyperlipidemia of enterocolitis. In miniature horses, hyperlipidemia may be secondary to enterocolitis. However, these horses normally may have increased incidence of hyperlipidemia.

f. Inappetence or starvation is often associated with hypertriglyceridemia, particularly in horses. This is likely due to increased mobilization of fat to meet energy demand and may be ameliorated with administration of parenteral nutrition or IV dextrose administration. Fasting hypertriglyceridemia occurs in dogs, and may be familial in Miniature Schnauzers and Beagles.

g. In parvovirus enteritis, hypertriglyceridemia may be seen, accompanied by hypocholesterolemia.

E. Lipemia is a term used for the milky appearance of serum or plasma caused by increased concentrations of triglyceride-carrying lipoproteins (i.e., chylomicra and VLDL). Increased concentrations of cholesterol do not cause lipemia.

1. Effects of lipemia on laboratory tests and management of the problem

a. Major effects of lipemia *in vitro*

(1) Lipemia enhances hemolysis, presumably due to a detergent effect on the cell membrane. Hemolyzed serum may alter test laboratory results (e.g., lipase activity is markedly inhibited by free hemoglobin).

(2) Lipemia-induced turbidity of the serum may impair end-point spectrophotometric determinations of selected serum analytes (e.g., glucose, calcium, phosphorus, total bilirubin values may be falsely high and total protein and albumin may be falsely low).

www.Ebook777.com

(3) Serum Na^+ and K^+ values measured in lipemic samples may be falsely low due to lipid displacement of the aqueous phase of serum. This problem occurs with flame photometers and certain ion-specific electrodes in which the serum specimen must be diluted prior to analysis. Values determined by ion-selective electrodes that require undiluted serum are clinically accurate in lipemic samples.

(4) Plasma protein values, measured by refractometry, may be falsely high. An indistinct line of demarcation on the protein scale usually is interpreted as an increased reading.

(5) Lipemia may interfere with hemoglobin determination, producing a falsely high value. Subsequently, calculation of the MCH and MCHC will be erroneous.

b. Management of lipemia for purposes of acquiring better laboratory specimens

(1) Fasting the patient for 24 hours usually eliminates lipemia.

(2) Lipemic serum may be refrigerated and the aliquot for analysis may be collected below the lipid layer.

(3) Ultracentrifugation or the use of polyethylene glycol 6000 as a precipitating agent may be used to clear lipemic serum.

(4) In cases in which lipemia persists after fasting, *in vivo* clearing of plasma lipids may be enhanced by administering intravenous sodium heparin (100 IU/kg in dogs) and collecting the blood sample 15 minutes later.

2. Causes of lipemia are listed in Table 6.1.

IV. OBESITY

A. Research pertaining to causes of obesity and metabolic consequences of obesity have identified associated perturbations in traditional blood parameters as well as additional factors that are involved in lipid metabolism.

1. In dogs, chronic obesity is associated with increases in total cholesterol (all fractions increased) and triglyceride concentrations. Obese cats have increased non-esterified fatty acids, triglycerides, and VLDL, with lower HDL. Obesity has been identified as a risk factor for developing diabetes in cats.

2. Adipokines are produced by adipocytes and/or macrophages and participate in a variety of functions including fat metabolism, cardiovascular dynamics and angiogenesis, inflammatory and immune responses, and hematopoiesis. Alterations in circulating values of adipokines are associated with a variety of co-morbidities seen in obesity, for example, insulin resistance. Some emerging factors whose circulating values are affected by obesity include the following:

a. Leptin is highly conserved across vertebrate species, promotes energy burning, and suppresses appetite. It also functions as a growth factor, suppresses apoptosis, and stimulates angiogenesis. Leptin commonly is increased in obese individuals, who develop leptin resistance; leptin levels correlate with body fat content. Circadian rhythm, proximity to feeding, gender, glucocorticoids, and stage of estrous cycle can influence circulating leptin levels at the time of measurement.

b. Adiponectin circulates in a number of forms of varying sizes; the high-molecular-weight (HMW) forms have the most biological activity. Some of the biological activities include anti-inflammatory properties, vasodilation, increasing insulin sensitivity, lowering blood glucose, and lowering tissue triglycerides. Adiponectin, particularly HMW adiponectin, is decreased in obesity.

c. Resistin (demonstrated in cattle, pigs, humans, and mice to date) is secreted primarily by adipocytes or macrophages, depending on the species, and increases with increasing body fat. Resistin is proinflammatory and contributes to insulin resistance.

PLASMA CARBOHYDRATES

Glucose metabolism and blood glucose concentration are abnormal in many diseases, and hyperglycemia and hypoglycemia are common laboratory findings. Disorders of glucose metabolism

Free ebooks ==> www.Ebook777.com

caused by diseases of the endocrine system, particularly the endocrine pancreas, are described in greater detail in Chapter 11. Ketones and lactate are organic anions that may be produced in excess during abnormal carbohydrate metabolism. Laboratory assessment of ketones and lactate is described in this section.

I. GLUCOSE

A. Sources of blood glucose

1. Dietary carbohydrate

a. After partial processing by salivary enzymes and stomach acid, the majority of carbohydrate hydrolysis occurs in the small intestine in monogastric animals. Pancreatic amylase digests carbohydrates to disaccharides. Mucosal disaccharidases subsequently digest disaccharides to monosaccharides (e.g., glucose, fructose, galactose), which are absorbed.

b. Monosaccharides are transported via portal blood to the liver where they are metabolized for energy, stored as glycogen, or converted to amino acids or fats.

c. Blood glucose increases for two to four hours postprandially in monogastric animals.

(1) A 12-hour fast should eliminate dietary carbohydrate ingestion as a cause of hyperglycemia.

(2) Postprandial hyperglycemia may be prolonged in hepatic disease because of reduced hepatocellular glycogenesis (storage of glucose as glycogen).

d. Ruminants have an absence of or minimal postprandial hyperglycemia because most of the dietary carbohydrates are fermented by microbes to volatile fatty acids (acetic, propionic, and butyric acids) in the rumen.

e. Starvation and malabsorption may cause hypoglycemia by restricting dietary intake and absorption of glucose, respectively (Case 16).

f. Birds have higher blood glucose values in health compared to mammals. Avian blood glucose values range from 209 to 399 mg/dL (see below).

2. Glycogenolysis

a. Glycogenolysis is the degradation of glucose.

b. Glycogen is the primary source of glucose during short periods of fasting. Depletion of liver glycogen stores occurs in many disease states that are characterized by a negative energy balance.

c. In the liver, glycogen is degraded to glucose that is released into the blood, resulting in hyperglycemia. Muscle glycogenolysis produces lactate and pyruvate, which are transported to the liver and converted (predominately lactate) to glucose.

d. Glycogenolysis is promoted by the following:

(1) Catecholamines (epinephrine)

(a) Catecholamines are released during fear, excitement, and muscular exertion (Cases 14, 18, 21, 22, 23).

(b) Epinephrine promotes glycogenolysis in hepatocytes and myocytes.

(c) Blood glucose concentration seldom exceeds 150 mg/dL in the dog, but values may exceed 300 mg/dL in the cat. Hyperglycemia may be accompanied by glucosuria in the cow or cat (Chapter 9).

(d) The mild hyperglycemia often seen with hyperthyroidism in cats may be partially mediated by increased sensitivity to epinephrine.

(e) Hyperglycemia and glucosuria seen in moribund cattle or cattle with neurological signs may be caused by catecholamine release.

(2) Glucagon (Case 14)

(a) Glucagon is released in response to hypoglycemia (low blood glucose).

(b) Glucagon only promotes hepatic glycogenolysis.

(c) Glucagon antagonizes the effect of insulin.

www.Ebook777.com

(3) Certain drugs may cause hyperglycemia. Examples include glucocorticoids, dextrose-containing parenteral fluids, thiazide diuretics, megesterol acetate, growth hormone, and morphine.

e. Glycogenolysis is inhibited in glycogen storage diseases; hypoglycemia may occur.

f. Glucocorticoids decrease glycogenolysis; therefore, hepatic glycogen stores are increased. Hypoglycemia does not occur, due to the hyperglycemic effects of glucocorticoids via other mechanisms, including gluconeogenesis (formation of glucose from protein or fat).

g. Reduced glycogen stores in hepatic disease may lead to fasting hypoglycemia.

h. Neonates have decreased glycogen stores and are prone to hypoglycemia during fasting.

3. Gluconeogenesis is the synthesis of glucose from amino acids and fats. Protein catabolism promotes gluconeogenesis and may be characterized by muscle wasting. Gluconeogenesis is promoted by the following:

a. Corticosteroids

(1) Excessive endogenous corticosteroid (cortisol) release in response to pain, extremes in body temperature, and other stressful events may lead to hyperglycemia in mammals (Cases 20, 22, 23).

(2) Hyperglycemia may be associated with hyperadrenocorticism (Case 26), while hypoglycemia may occur with hypoadrenocorticism.

(3) Corticosteroids are antagonistic to insulin and decrease uptake and use of glucose by tissues.

b. Glucagon

c. Growth hormone

d. Certain drugs (see above)

B. Fate of blood glucose

1. Glycogenesis

a. Insulin shifts glucose metabolism in the liver and muscle toward glycogen synthesis.

2. Tissue utilization

a. Cellular uptake of glucose is stimulated by insulin.

(1) Muscle, liver, and fat are the major tissues affected.

(2) Insulin is not as necessary for glucose uptake by erythrocytes, neurons, and renal tubular epithelial cells.

(3) Blood glucose concentrations greater than 90 mg/dL usually stimulate insulin release.

b. Increased tissue use of glucose and hypoglycemia may occur with the following:

(1) Excess administration or secretion of insulin or insulin analogs

(a) Iatrogenic insulin overdose

(b) β-cell tumor (insulinoma, islet cell tumor) of the pancreatic islets of Langerhans

(c) Paraneoplastic syndromes (e.g., leiomyoma, leiomyosarcoma)

(2) Reduction of hormones that maintain glucose homeostasis

(a) Hypoadrenocorticism

(3) Reduced hepatic storage of glycogen

(a) Advanced liver disease

(b) Sepsis due to Gram-negative endotoxemia and Gram-positive anaerobes

(c) Glycogen storage diseases

(4) Excessive use of glucose

(a) Sepsis

(b) Extreme physical exertion (hunting dogs)

(c) Pregnancy

(d) Polycythemia

(e) Extreme leukocytosis

(f) Neoplasia

Free ebooks ==> www.Ebook777.com

(5) Reduced glucose intake or inadequate gluconeogenesis
(a) Neonates (especially toy breeds of dogs)
(b) Severe malnutrition
(c) Severe malabsorption
(d) Starvation
(6) Prolonged contact of serum with erythrocytes *in vitro*. Failure to remove the serum from the clot or to separate plasma from blood cells is the most common cause of pseudohypoglycemia in clinical laboratory specimens.

c. Decreased tissue use of glucose with subsequent hyperglycemia may occur with the following:
(1) Insulin antagonists that increase peripheral resistance of tissues to the effects of insulin
(a) Exogenous corticosteroid administration
(b) Excessive endogenous cortisol (mammals) or corticosterone (birds) production and/or release
(c) Glucagon
(d) Growth hormone
(2) Diabetes mellitus (Case 15)
(a) Type I (type 1) diabetes with decreased insulin production
(b) Type II (type 2) diabetes with delayed or inadequate insulin secretion
(3) Increased tissue breakdown and carbohydrate metabolism caused by hyperthyroidism

C. Maintenance of blood glucose concentration
1. Glucose concentration is governed by multiple factors that affect entry and removal of glucose from the blood.
2. A balance between glucose absorption, insulin production, and insulin antagonists (glucagon, corticosteroids, growth hormone) maintains appropriate blood glucose concentrations in health.

D. Glucose metabolism in birds
1. Basic glucose metabolism in birds is similar to that in mammals.
2. Fasting blood glucose values (209 to 399 mg/dL) in birds are considerably higher than those of mammals.
3. The avian pancreas has lower insulin and higher glucose stores than does the mammalian pancreas.
4. Plasma glucagon concentration is higher in birds than in mammals.

E. Means of evaluating glucose metabolism
1. Blood glucose concentration
a. Proper sample management is imperative to correctly interpret blood glucose values.
(1) Sampling of frightened animals should be avoided; excessive catecholamine release may cause hyperglycemia.
(2) If the medical history precludes the likelihood of hypoglycemia, nonruminants and very young ruminants should be fasted at least 12 hours to avoid postprandial hyperglycemia.
(3) If the medical history or clinical signs (e.g., weakness, muscle twitching, depression, seizures) suggest hypoglycemia, fasting of the patient should be avoided. A non-fasting, baseline glucose concentration is determined.
(a) If the baseline glucose value is within the reference interval, the patient is fasted and blood glucose concentrations are determined every two hours.
(b) Hypoglycemia is confirmed if the blood glucose concentration falls below 40 mg/dL, whereas glucose values greater than 50 mg/dL after a 24-hour fast are less suggestive of hypoglycemia.
(4) Serum or plasma should be separated from blood cells (i.e., the clot in a specimen without anticoagulant) if more than 30 minutes will lapse between sample collection and assay.
(a) In vitro glycolysis is the most common cause of pseudohypoglycemia in laboratory specimens.

www.Ebook777.com

(b) In vitro glycolysis by blood cells decreases blood glucose concentration approximately 10%/hour at room temperature.

(c) An alternative to serum or plasma separation is use of an anticoagulant solution containing sodium fluoride to inhibit glycolysis. Sodium fluoride specifically inhibits enolase, an enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway. Sodium fluoride also inhibits some diagnostic enzymes. If the anticoagulant is potassium oxalate, glucose measurement may be artifactually decreased.

b. Lipemia markedly interferes with the accuracy of glucose measurement in some assays (e.g., end-point colorimetric tests). It is often present in diseases characterized by hyperglycemia (e.g., diabetes mellitus). The management of lipemic specimens is described earlier in this chapter (see Plasma Lipids).

c. Assay techniques are primarily spectrophotometric using colorimetric end-point or kinetic assays.

(1) Colorimetric end-point assays are inaccurate with lipemic specimens; decreased light transmittance accompanies sample turbidity.

(2) Kinetic assays are enzymatic and self-blanking. They detect changes in optical density of the specimen over time. The laboratory results are more accurate with this type of assay.

d. Colorimetric reagent strips, read visually or with a reflectance meter, are available for estimating glucose concentration in fresh whole blood.

(1) The strips may reliably detect hyperglycemia, but hypoglycemia may be missed.

(2) Proper controls should be run if point-of-care analyzers are used; the instrument should be validated and maintained to provide accurate results.

(3) Anemia may result in overestimation of blood glucose concentrations.

(4) The accuracy of abnormal glucose values, determined by colorimetric reagent strips, should be verified before clinical decisions are made regarding treatment.

e. Portable blood glucose meters have a place in daily monitoring of diabetics, but they are not always accurate for veterinary specimens, particularly at higher concentrations of glucose.

f. Continuous glucose monitoring devices have been used in dogs, cats, and horses, and greatly refine the assessment of diabetes control. These devices record interstitial glucose concentrations, which correlate with blood glucose. By recording values frequently (typically every five minutes), fluctuations and response to insulin, feeding, etc. can be monitored. In addition, the assessments can be done in the animal's normal environment, which minimizes handling, caging, and transport stress-related effects on blood glucose. Additional uses for the devices include managing ketoacidotic diabetic patients and other critical care patients with hypo- or hyperglycemia in a hospital setting.

2. Urine glucose (see Chapter 9)

a. The resorptive capacity of the proximal renal tubule is exceeded when blood glucose concentration exceeds 100 mg/dL in the cow, 180 mg/dL in the dog, 280 mg/dL in the cat, and 600 mg/dL in birds.

b. In glucosuria, urine-specific gravity increases 0.004 g/dL of glucose present. A 4+ reagent strip reading represents approximately 2 g/dL of glucose in the urine.

c. Because urine collects in the bladder over time, previous transient hyperglycemia results in mild glucosuria, even though the blood glucose concentration has returned to the reference interval.

3. Serum insulin and amended insulin/glucose ratios (Chapter 11, Endocrine Pancreas). These measurements and calculations are used to detect hyperinsulinism; they have generally replaced glucose tolerance tests for this purpose

4. Glucose tolerance tests

a. Indications

(1) Intravenous or oral glucose tolerance tests may be used to detect hyperinsulinism.

Free ebooks ==> www.Ebook777.com

(2) Intravenous or oral glucose tolerance tests also may be used to detect glucose intolerance in animals with persistent hyperglycemia below the renal threshold. Once persistent hyperglycemia exceeds the renal threshold, glucose tolerance testing adds no further diagnostic information.

(3) For patients in which diabetes mellitus has been confirmed, the intravenous glucose tolerance test, combined with the measurement of serum insulin after the glucose injection, may be used to identify the type of diabetes mellitus (Chapter 11). The type of diabetes is identified by the insulin response pattern.

(4) The most common use of the oral glucose tolerance test is to evaluate intestinal absorption.

b. Dosages and interpretive guidelines. Blood glucose concentration is measured after a 12-hour fast (if the animal is not hypoglycemic) and at appropriate intervals after glucose administration.

(1) Oral glucose tolerance test

(a) Dogs are given 1.75 g glucose/kg body weight per os. Horses are given 1 g glucose/kg as a 20% solution via stomach tube. Oral glucose tolerance tests cannot be performed in ruminants because ruminal microflora ferment ingested carbohydrates.

(b) Blood samples for glucose measurement are collected at 30-minutes intervals.

(c) The blood glucose concentration should approach 160 mg/dL by 30 to 60 minutes post glucose administration and return to baseline values by 120 to 180 minutes in normal dogs. In horses, the blood glucose concentration should approach 175 mg/dL by 120 minutes and return to baseline values within 360 minutes.

(d) Failure to reach these maximal blood glucose concentrations suggests intestinal malabsorption, delayed gastric emptying, or vomiting. Increased glucose tolerance and lower than expected peak values may be seen in hypothyroidism, adrenal insufficiency, hypopituitarism, and hyperinsulinism.

(e) Failure of the blood glucose concentration to return to baseline values within the expected time suggests glucose intolerance, which is a feature of diabetes mellitus, hyperadrenocorticism, hyperthyroidism, and hepatic insufficiency. Impaired glucose tolerance also may occur as a consequence of high fat content of the diet.

(f) After glucose concentrations reach baseline values, a brief hypoglycemic phase occurs. The glucose concentration subsequently returns to baseline as glucagon is released. Severe hypoglycemia may occur if glucagon secretion is impaired.

(2) Intravenous glucose tolerance test

(a) After a 12-hour fast (ruminants do not require fasting), a baseline blood glucose specimen is drawn and then glucose is administered. In dogs and cats, 1 g glucose/kg is given intravenously over a 30-second period. In large animals, the calculated dosage of glucose is given within two to three minutes. Timing of the test begins at the midpoint of glucose injection. Blood samples are collected at 5, 15, 25, 35, 45, and 60 minutes after administration. In cats, an additional blood sample is collected at 120 minutes.

(b) Using semilog graph paper, the glucose values are plotted on log scale vs. time on the arithmetic scale. The time required for the glucose value to decrease by 50% is determined from the graph (usually 45 minutes or less in the dog).

(c) Prolonged glucose disappearance indicates intolerance (the same diseases as described for the oral test above).

(d) Insulin response also can be assessed; the peak insulin concentration should occur within 15 minutes of glucose administration with a return to baseline values at 60 minutes in most species, 120 minutes in the cat. Insulin response is most useful in differentiating types of diabetes mellitus (Chapter 11).

(e) Glucose clearance is enhanced and circulating insulin concentrations (both pre- and post-glucose injection) are decreased in horses with polysaccharide storage myopathy.

www.Ebook777.com

5. Glycated proteins
   a. Glucose molecules irreversibly bind to proteins, providing a means of assessing long-term blood glucose concentrations.
   b. Glycated protein assays include fructosamine (FrAm), hemoglobin Alc (HbAlc), and glycoalbumin (Galb).
   c. Fructosamine is an amino sugar formed by the reduction of the osazone glucosamine. Fructosamine quantitation is the most commonly used glycated protein assay in veterinary medicine. This colorimetric test is based on the reduction of fructosamine. In this assay, the glycated proteins are albumin and other serum proteins. Their measurement allows a determination of average blood glucose over the previous two to three weeks, which roughly corresponds to the protein half-life.
      (1) Fructosamine measurement is useful in diagnosing diabetes mellitus, differentiating diabetes mellitus from stress-induced hyperglycemia in cats or renal glucosuria, and monitoring treatment of diabetics. Fructosamine concentration is increased with chronic hyperglycemia, but is within the reference interval in transient hyperglycemia.
      (2) There is considerable inter-individual variability in fructosamine in hyperglycemic cats, resulting in a wide range irrespective of the degree of hyperglycemia. Serial fructosamine measurements in individual cats to monitor diabetic control are more useful than estimation of mean glucose concentration from a single plasma fructosamine concentration.
      (3) Normal fructosamine and glucose values vary between psittacine species; therefore, species-specific reference intervals are critical. Fructosamine values can be used to identify and monitor diabetic psittacines; however, fructosamine values increase and decrease more rapidly than in mammals, resulting in fructosamine increases in transient hyperglycemia and decreases similar to those of glucose after insulin administration. Thus, fructosamine is less useful for long-term monitoring of blood glucose in these species.
      (4) Hypoproteinemia (cats) and/or hypoalbuminemia may result in decreased fructosamine concentrations.
      (5) If albumin and protein values are within the reference interval and fructosamine values are below the reference interval, the patient should be evaluated further for hyperinsulinism. Insulinoma has presented with normal fasting glucose concentrations and low fructosamine values.
   d. Glycoalbumin measurement has been demonstrated as an acceptable alternative to fructosamine assessment in dogs and cats.
   e. HbA1c concentration is measured less frequently. Its concentration reflects the blood glucose concentration over the previous two to three months.
   f. Glycated hemoglobin measurement has been used to assess resource intake, growth, and body condition of birds.
6. Less commonly used diagnostic tools to evaluate glucose metabolism
   a. Insulin tolerance and glucagon stimulation tests have been used to evaluate carbohydrate metabolism.
   b. Epinephrine's ability to promote glycogenolysis and increase blood glucose concentration has been exploited to assess liver glycogen stores.
7. Beta-cell function in cats can be determined using immunoradiometric or enzyme-linked immunosorbent methods to measure proinsulin and determine proinsulin:insulin ratios in blood. Proinsulin is converted to insulin by beta cells.

F. Hyperglycemic disorders (Cases 14, 15, 18, 20, 21, 22, 23, 26, 27). Diseases and circumstances associated with persistent or transient hyperglycemia and glucosuria are listed in Table 6.2.

G. Hypoglycemic disorders (Case 16). Diseases and circumstances associated with hypoglycemia are listed in Table 6.3.

Free ebooks ==> www.Ebook777.com

TABLE 6.2.
CAUSES OF HYPERGLYCEMIA.

- Persistent hyperglycemia
  - Acromegaly
  - Diabetes mellitus
  - Hyperadrenocorticism
  - Hyperglucagonemia
  - Hyperpituitarism
  - Hyperthyroidism
  - Pheochromocytoma
- Transient hyperglycemia
  - Acute pancreatitis
  - Acute severe colic
  - Ammonia toxicosis
  - Bovine displaced abomasum
  - Bovine milk fever
  - Bovine neurologic diseases
  - Diestrus
  - Drugs and chemicals
    - ACTH
    - Corticosteroids
    - Detomidine
    - Ethylene glycol toxicosis
    - Glucose-containing parenteral fluids
    - Halothane (prolonged anesthesia in horses)
    - Ketamine
    - Megestrol acetate
    - Morphine
    - Phenothiazine
    - Progestogens
    - Thiazide diuretics
    - Xylazine
  - Equine acute abdominal disease
  - Fear, excitement, exertional catecholamine release (especially cats)
  - Head trauma (dogs, cats)
  - Hyperthyroidism
  - Moribund animals (particularly cattle)
  - Ovine enterotoxemia
  - Ovine listeriosis
  - Ovine transport tetany
  - Postprandial
  - Stress, endogenous corticosteroids

II. KETONES

A. Basic concepts

1. The ketone bodies (anions of buffered ketoacids) are acetoacetate, β-hydroxybutyrate, and acetone. The former two ketones are intermediary metabolites of lipid metabolism, while the latter is a waste product.

www.Ebook777.com

TABLE 6.3.
CAUSES OF HYPOGLYCEMIA.

Excess administration or secretion of insulin and insulin analogs
- Beta-cell tumor (insulinoma, islet cell tumor) of the pancreas
- Extrapancreatic neoplasms, including those with possible secretion of abnormal insulin-like growth factor (paraneoplastic syndrome)
  - Hemangiosarcoma
  - Hepatocellular carcinoma
  - Leiomyoma, leiomyosarcoma
  - Melanoma
  - Renal carcinoma
  - Salivary adenocarcinoma
- Iatrogenic insulin overdose

Reduction of hormones that maintain glucose homeostasis
- Hypoadrenocorticism
- Hypopituitarism
- Hypothyroidism

Reduced hepatic storage of glycogen
- Advanced liver disease/hepatic insufficiency (paradoxically, prolonged postprandial hyperglycemia and glucose intolerance)
- Aflatoxicosis in horses
- Fat cow syndrome
- Glycogen storage diseases
- Hyperlipidemia in ponies
- Sepsis (Gram-negative endotoxemia and Gram-positive anaerobes)

Increased use of glucose
- Extreme leukocytosis
- Extreme physical exertion (hunting dogs, endurance-ride horses)
- *In vitro* glycolysis (common cause of pseudohypoglycemia)
- Neoplasia (paraneoplastic syndrome)
- Polycythemia
- Pregnancy toxemia in sheep, dogs, cows, and goats
- Sepsis (Gram-negative endotoxemia and Gram-positive anaerobes)

Reduced glucose intake or inadequate gluconeogenesis
- Juvenile hypoglycemia (piglets, toy and miniature breeds of dogs)
- Ketosis in cattle
- Malabsorption syndromes
- Neonatal hypoglycemia
- Severe malnutrition
- Starvation

Drug effects causing hypoglycemia
- Ethanol
- Insulin overdose
- o,p′-DDD
- Salicylates
- Sulfonylurea

Free ebooks ==> www.Ebook777.com

2. Ketone production always increases when carbohydrate depletion and increased gluconeogenesis occur. In these circumstances, oxaloacetate depletion in the Krebs cycle and suppressed lipogenesis lead to acetoacetyl coenzyme A production, the precursor to ketogenesis.

3. Routine laboratory tests usually detect ketonuria before ketonemia can be identified.

a. The renal threshold for ketones is low. Following glomerular filtration, ketones are incompletely reabsorbed by the renal tubular epithelial cells, resulting in ketonuria. Ketonuria is specifically detected by dipstick analysis via the nitroprusside reaction (Chapter 9).

b. Routine biochemical profiles do not specifically detect ketones. Their presence in serum is suggested indirectly by titration acidosis (decreased HCO_3^- or TCO_2) and an increased anion gap. However, lactic acidosis can produce similar laboratory abnormalities (chapters 5 and 9).

4. Ketones can provide an important alternate energy source to numerous tissues. Their production is a survival mechanism in situations of energy deficit. The highly acidic nature of acetoacetate and β-hydroxybutyrate often results in the clinical syndrome of ketoacidosis if they are present in high concentrations in the plasma (Chapters 5 and 11).

B. Measurement of ketones

1. Most qualitative tests detect ketones by the nitroprusside reaction. This reaction is specific for acetoacetate and to a lesser extent acetone; β-hydroxybutyrate is not detected. Because β-hydroxybutyrate is a major ketone in diabetic ketoacidosis, the degree of intensity of the nitroprusside reaction does not correlate with the severity of ketosis. Beta-hydroxybutyrate strips have been developed for use in milk; however, reports of their advantages over the nitroprusside method in diagnosing bovine ketosis are conflicting.

2. Specimens of milk, urine, or serum for ketone quantitation should be fresh to avoid false-negative reactions following the degradation of acetoacetate:

a. Milk may be tested directly.

b. Urine should be diluted 1:10 with distilled water to avoid false-positive reactions on urine reagent strips.

c. Serum should be diluted 1:1 with water to avoid false-positive reactions.

3. An enzymatic and colorimetric method has been developed for quantification of β-hydroxybutyrate. Consult the laboratory performing the test for specimen handling instructions.

4. Several drugs and endogenous substances can cause false positive reactions in urine.

5. Ketonemia may be suspected when the calculated anion gap (Chapter 5) is increased. Diabetic ketonemia is primarily due to β-hydroxybutyrate, which does not react with the nitroprusside reagents. Therefore, a negative serum ketone test does not exclude ketonemia as the cause of an increased anion gap.

C. Causes of ketonemia and ketonuria

1. Anorexia, starvation, diabetes mellitus (Case 15), bovine ketosis, displaced abomasum, ovine pregnancy toxemia, equine hyperadrenocorticism, high-fat diet, and bovine hepatic lipidosis syndrome are the major causes of ketonemia and ketonuria.

2. Lactation in dairy goats and pregnancy in dogs, goats, and cattle (particularly with multiple fetuses) may cause ketosis.

3. Heavy exercise in dogs and horses may result in a negative energy balance with development of ketosis. Increased release of fatty acids into the plasma and subsequent conversion of the fatty acids to ketones by the liver causes ketosis.

4. The ketonemia of diabetes mellitus is accompanied by hyperglycemia, whereas ketonemia of other causes usually is accompanied by normoglycemia or hypoglycemia.

III. LACTATE

A. Basic concepts

1. Lactate is the organic anion of buffered lactic acid. It is produced in excess during anaerobic metabolism.

www.Ebook777.com

2. Endogenous lactic acid production increases as tissue perfusion and oxygenation decrease. Lactic acid diffuses immediately into extracellular fluid and dissociates into hydrogen ion and lactate.

3. Enteric organisms increase exogenous lactic acid production during carbohydrate overload and occasionally with diarrhea. Absorbed lactic acid also dissociates into hydrogen ion and lactate.

B. Measurement of lactate

1. Blood samples for quantitative lactate analysis must be collected into sodium fluoride-containing anticoagulant, which prevents *in vitro* glycolysis and increased lactate production. A test method using a portable hand-held meter has been developed for point-of-care evaluation of lactate concentrations.

2. Most test methods use L-lactate dehydrogenase. This enzymatic assay detects L-lactate, but does not detect D-lactate (Chapter 5). D-lactate can be measured by gas-liquid chromatography or D-lactate dehydrogenase methods.

3. Increased serum lactate concentration may be suspected when the calculated anion gap (Chapter 5) is increased and clinical circumstances are consistent with lactic acid production.

4. Serum lactate measurement may be used to determine exercise tolerance in assessing adequacy of cardiac function.

C. Causes of hyperlactatemia include shock, grain overload, sustained heavy exercise, diabetic ketoacidosis, and colic in horses that is characterized by torsion, strangulation, or rupture. Patient prognosis is poor when blood lactate concentrations are very high.

REFERENCES

Adin DB, Oyama MA, Sleeper MM, et al: 2006. Comparison of canine cardiac troponin I concentrations as determined by 3 analyzers. *J Vet Intern Med* 20:1136–1142.

Apple FS, Murakami MM, Ler R, et al: 2008. Analytical characteristics of commercial cardiac troponin I and T immunoassays in serum from rats, dogs, and monkeys with induced acute myocardial injury. *Clin Chem* 54:1982–1989.

Ardia DR: 2006. Glycated hemoglobin and albumin reflect nestling growth and condition in American kestrels. *Comp Biochem Physiol* 143:62–66.

Baker JL, Aleman M, Madigan J: 2001. Intermittent hypoglycemia in a horse with anaplastic carcinoma of the kidney. *J Am Vet Med Assoc* 218:235–237.

Baker RJ, Valli VEO: 1986. A review of feline serum protein electrophoresis. *Vet Clin Pathol* 15:20–25.

Barrie J, Nash AS, Watson TDG: 1993. Quantitative analysis of canine plasma lipoproteins. *J Small Anim Pract* 34:226–231.

Barrie J, Watson TDG, Stear MJ, et al: 1993. Plasma cholesterol and lipoprotein concentrations in the dog: The effects of age, breed, gender and endocrine disease. *J Small Anim Pract* 34:507–512.

Batamuzi EK, Kristensen F, Jensen AL: 1996. Serum protein electrophoresis: Potential test for use in geriatric companion animal health programmes. *J Vet Med*, Series A 43:501–508.

Bauer JE: 2004. Lipoprotein-mediated transport of dietary and synthesized lipids and lipid abnormalities of dogs and cats. *J Am Vet Med Assoc* 224:668–675.

Bauer JE: 1992. Diet-induced alterations of lipoprotein metabolism. *J Am Vet Med Assoc* 201:1691–1694.

Bauer JE, Brooks TP: 1990. Immunoturbidimetric quantification of serum immunoglobulin G concentration in foals. *Am J Vet Res* 51:1211–1214.

Free ebooks ==> www.Ebook777.com

Bauer JE, Meyer DJ, Campbell M, et al: 1990. Serum lipid and lipoprotein changes in ponies with experimentally induced liver disease. *Am J Vet Res* 51:1380–1384.

Blanchard G, Paragon BM, Serougne C, et al: 2004. Plasma lipids, lipoprotein composition and profile during induction and treatment of hepatic lipidosis in cats and the metabolic effect of one daily meal in healthy cats. *J Anim Phys and Anim Nutr* 88:73087.

Boswood A: 2009. Biomarkers in cardiovascular disease: Beyond natriuretic peptides. *J Vet Cardiology* 11:S23–32.

Boswood A: 2004. Editorial: The rise and fall of the cardiac biomarker. *J Vet Intern Med* 18:797–799.

Bruss ML: 1997. Lipids and Ketones. *In*: Kaneko JJ, Harvey JW, Bruss ML (eds): Clinical Biochemistry of Domestic Animals, 5th ed. San Diego, Academic Press, pp. 83–115.

Burgener IA, Kovacevic A, Mauldin GN, et al: 2006. Cardiac troponins as indicators of acute myocardial damage in dogs. *J Vet Intern Med* 20:277–283.

Caldin M, Tasca S, Carli E, et al: 2009. Serum acute phase protein concentrations in dogs with hyperadrenocorticism with and without concurrent inflammatory conditions. *Vet Clin Pathol* 38:63–8.

Ceciliani F, Grossi C, Giordano A, et al: 2004. Decreased sialylation of the acute phase protein α_1-acid glycoproteinin feline infectious peritonitis (FIP). *Vet Immunol Immunopathol* 99:229–236.

Ceron JJ, Eckersall PD, Martinez-Subiela S: 2005. Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet Clin Pathol* 34:85–99.

Christopher MM: 1997. Hyperlipidemia and other clinicopathologic abnormalities associated with canine hypothyroidism. *Canine Pract* 22:37–38.

Christopher MM, O'Neill S: 2000. Effect of specimen collection and storage on blood glucose and lactate concentrations in healthy, hyperthyroid and diabetic cats. *Vet Clin Pathol* 29:22–28.

Clabough DL, Conboy HS, Roberts MC: 1988. Comparison of four screening techniques for the diagnosis of equine neonatal hypogammaglobulinemia. *J Am Vet Med Assoc* 194:1717–1720.

Cohn LA, McCaw DL, Tate DJ, et al: 2000. Assessment of five portable blood glucose meters, a point-of-care analyzer, and color test strips for measuring blood glucose concentration in dogs. *J Am Vet Med Assoc* 216:198–202.

Conner JG, Eckersall PD: 1986. Acute phase response and mastitis in the cow. *Res Vet Sci* 41:126–128.

Connolly DJ, Cannata J, Boswood A, et al: 2003. Cardiac troponin I in cats with hypertrophic cardiomyopathy. *J Feline Medicine and Surgery* 5:209–216.

Connoly DJ, Guitian J, Boswood A, et al: 2005. Serum troponin I levels in hyperthyroid cats before and after treatment with radioactive iodine. *J Feline Medicine and Surgery* 7:289–300.

Cook NB, Ward WR, Dobson H: 2001. Concentrations of ketones in milk in early lactation, and reproductive performance of dairy cows. *Vet Rec* 148:769–772.

Couto CG, Ceron JJ, Parra MD, et al: 2009. Acute phase protein concentrations in retired racing greyhounds. *Vet Clin Pathol* 38:219–23.

Cray C, Rodrigueaz M, Zaias J: 2007. Protein electrophoresis of psittacine plasma. *Vet Clin Pathol* 36:64–72.

Crenshaw KL, Peterson ME, Heeb LA, et al: 1996. Serum fructosamine concentration as an index of glycemia in cats with diabetes mellitus and stress hyperglycemia. *J Vet Intern Med* 10:360–364.

Crispin SM, Bolton CH, Downs LG: 1992. Plasma lipid and lipoprotein profile of working and pet border collies. *Vet Rec* 130:185–816.

www.Ebook777.com

DeBowes LJ: 1987. Lipid metabolism and hyperlipoproteinemias in dogs. *Compend Contin Educ Pract Vet* 9:727–736.

DeFrancesco TC, Rush JE, Rozanski EA, et al: 2007. Prospective clinical evaluation of an ELISA B-type natriuretic peptide assay in the diagnosis of congestive heart failure in dogs presenting with cough or dyspnea. *J Vet Intern Med* 21:243–259.

De La Corte FD, Valberg SJ, MacLeay JM, et al: 1999. Glucose uptake in horses with polysaccharide storage myopathy. *Am J Vet Res* 60:458–462.

Diehl KJ, Lappin MR, Jones RL, et al: 1992. Monoclonal gammopathy in a dog with plasmacytic gastroenterocolitis. *J Am Vet Med Assoc* 201:1233–1236.

Dorfman M, Dimski DS: 1992. Paraproteinemias in small animal medicine. *Compend Contin Educ Pract Vet* 14:621–632.

Downs LG, Bolton CH, Crispin SM, et al: 1997. Plasma lipoprotein lipids in five different breeds of dogs. *Res Vet Sci* 54:63–67.

Downs LG, Zani V, Wills JM, et al: 1994. Changes in plasma lipoprotein during the oestrous cycle of the bitch. *Res Vet Sci* 56:82–88.

Duncan JR, Prasse KW, Mahaffey EA: 1994. Proteins, Lipids, and Carbohydrates. *In*: Duncan JR, Prasse KW, Mahaffey EA (eds): Veterinary Laboratory Medicine: Clinical Pathology, 3rd ed. Ames, Iowa State University Press, pp. 112–129.

Dunkel B, McKenzie HC: 2003. Severe hypertriglyceridaemia in clinically ill horses: Diagnosis, treatment, and outcome. *Equine Vet J* 35:590–595.

Eckersall PD, Duthie S, Toussaint MJM, et al: 1999. Standardization of diagnostic assays for animal acute phase proteins. *Adv Vet Med* 41:643–55.

Eckersall PD, Saini PK, McComb C: 1996. The acute phase response of acid soluble glycoprotein, alpha-1-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. *Vet Immunol Immunopathol* 51:377–385.

Ford RB: 1993. Idiopathic hyperchylomicronaemia in miniature schnauzers. *J Small Anim Pract* 34:488–492.

Frank N, Sojka JE, Latour MA: 2002. Effect of withholding feed on concentration and composition of plasma very low density lipoprotein and serum nonesterified fatty acids in horses. *Am J Vet Res* 63:1018–1021.

Garry F, Adams R, Aldridge B: 1993. Role of colostral transfer in neonatal calf management: Current concepts in diagnosis. *Compend Contin Edu* 15:1167–1176.

Gascoyne SC, Bennett PM, Kirkwood JK, et al: 1994. Guidelines for the interpretation of laboratory findings in birds and mammals with unknown reference ranges: plasma biochemistry. *Vet Rec* 134:7–11.

Gebhardt C, Hirschberger J, Rau S, et al: 2009. Use of C-reactive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. *J Vet Emergency and Critical Care* 19:450–8.

Geishauser T, Leslie K, Kelton D, et al: 1998. Evaluation of five cowside tests for use with milk to detect subclinical ketosis in dairy cows. *J Dairy Sci* 81:438–443.

George JW: 2001. The usefulness and limitations of hand-held refractometers in veterinary laboratory medicine: An historical and technical review. *Vet Clin Pathol* 30:201–210.

Free ebooks ==> www.Ebook777.com

German AJ, Hervera M, Hunter L, et al: 2009. Improvement in insulin resistance and reduction in plasma inflammatory adipokines after weight loss in obese dogs. *Domestic Animal Endocrinology* 37:214–226.

Giraudel JM, Pages JP, Guelfi JF: 2002. Monoclonal gammopathies in the dog: A retrospective study of 18 cases (1986–1999) and literature review. *J Am Anim Hosp Assoc* 38:135–147.

Gonzales FHD, Tecles F, Martinez-Subiela S, et al: 2008. Acute phase protein response in goats. *J Vet Diagn Invest* 20:580–584.

Grau-Roma L, Heegaard MPH, Hjulsager CK, et al: 2009. Pig—major acute phase protein and haptoglobin serum concentrations correlate with PCV2 viremia and the clinical course of postweaning multisystemic wasting syndrome. *Vet Microbiol.* 138:53–61.

Greeve J, Altkemper I, Dieterich J-H, et al: 1993. Apolipoprotein B mRNA editing in 12 different mammalian species: hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. *J Lipid Res* 34:1367–1383.

Grosche A, Schrodl W, Schusser GF: 2006. Specific parameters of blood and peritoneal fluid to indicate the severity of intestinal ischaemia in colic horses. *Tierarztliche Praxix* 34:387–396.

Gruys E, Petersen B, Piniero C, Sankari S, Fuerll M, Paltrinieri S, McDonald T, O'Brien P, Molenaar A, Lampreave F, Heegaard P, Godeau J, Ceron J, Evans G, Hoff B, Torgerson P: 2005. The 5th International Colloquium on Animal Acute Phase Proteins Abstracts and Proceedings Book. Dublin, Ireland March 14–15.

Gutierrez AM, Martinez-Subiela S, Soler L, et al: 2009. Use of saliva for haptoglobin and C-reactive protein quantifications in porcine respiratory and reproductive syndrome affected pigs in field conditions. *Vet Immunol Immunopathol* 132:218–23.

Hall WF, Eurell TE, Hansen RD, et al: 1992. Serum haptoglobin concentration in swine naturally or experimentally infected with *Actinobacillus pleuropneumoniae*. *J Am Vet Med Assoc* 201:1730–1733.

Higai K, Aoki Y, Azuma Y, Matsumoto K: 2005. Glycosylation of site-specific glycans of alpha1-acid glycoprotein and alterations in acute and chronic inflammation. *Biochimica et Biophysica Acta* 1725:128–35.

Herndon WE, Kittleson MD, Sanderson K, et al: 2002. Cardiac troponin I in feline hypertrophic cardiomyopathy. *J Vet Intern Med* 16:558–565.

Herndon WE, Rishniw M, Schrope D, et al: 2008. Assessment of plasma cardiac troponin I concentration as a means to differentiate cardiac and noncardiac causes of dyspnea in cats. *J Am Vet Med Assoc* 233:1261–1264.

Hirvonen J, Pyorala S: 1998. Acute-phase response in dairy cows with surgically-treated abdominal disorders. *Vet J* 155:53–61.

Hoenig M, Wilkins C, Olson JC, et al: 2003. Effects of obesity on lipid profiles in neutered male and female cats. *Am J Vet Res* 64:299–303.

Holbrook TC, Birks EK, Sleeper MM, et al: 2006. Endurance exercise is associated with increased plasma cardiac troponin I in horses. *Equine Vet J* 36:27–31.

Hollis AR, Boston RC, Corley KTT: 2007. Blood glucose in horses with acute abdominal disease. *J Vet Intern Med* 21:1099–1103.

Hulten C, Sandgren B, Skioldebrand E, et al: 1999. The acute phase protein serum amyloid A (SAA) as an inflammatory marker in equine influenza virus infection. *Acta Vet Scand* 40:323–333.

Ileri-Buyukoglu T, Guldur T: 2005. Dyslipoproteinemias and their clinical importance in several species of domestic animals. *J Am Vet Med Assoc* 227:1746–1751.

www.Ebook777.com

Itoh H, Horiuchi Y, Nagasaki T, et al: 2009. Evaluation of immunological status in tumor-bearing dogs. *Vet Immunol and Immunopathol* 32:85–90.

Itoh N, Koiwa M, Hatsugaya A, et al: 1998. Comparative analysis of blood chemical values in primary ketosis and abomasal displacement in cows. *Zentralbl Veterinarmed* 45:293–298.

Jacobsen S, Kjelgaard-Hansen M: 2008. Evaluation of a commercially available apparatus for measuring the acute phase protein serum amyloid A in horses. *Vet Rec* 163:327–330.

Jacobsen S, Kjelgaard-Hansen M, Hagbard Petersen H, et al: 2006. Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet J* 172:315–319.

Jacobsen S, Thomas MH, Nanni S: 2006. Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease. *Am J Vet Res* 67:1738–1742.

Jensen AL: 1995. Glycated blood proteins in canine diabetes mellitus. *Vet Rec* 137:401–405.

Jeusette IC, Lhoest ET, Istasse LP, et al: 2005. Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. *Am J Vet Res* 66:81–86.

Jones BR: 1993. Inherited hyperchylomicronaemia in the cat. *J Small Anim Pract* 34:493–499.

Jones PA, Bain FT, Byars TD, et al: 2001. Effect of hydroxyethyl starch infusion on colloid oncotic pressure in hypoproteinemic horses. *J Am Vet Med Assoc* 218:1130–1135.

Jordan E, Kley S, Le N-A, et al: 2008. Dyslipidemia in obese cats. *Domestic Anim Endocrinol* 35:290–299.

Jorritsma R, Jorritsma H, Schukken YH, et al: 2001. Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial dairy herds in the Netherlands. *Livestock Prod Sci* 68:53–60.

Kajikawa T, Furuta A, Onishi T, et al: 1996. Enzyme-linked immunosorbent assay for detection of feline serum amyloid A protein by use of immunological cross-reactivity of polyclonal anti-canine serum amyloid A protein antibody. *J Vet Med Sci* 58:1141–1143.

Kaneko JJ: 1997. Carbohydrate Metabolism and its Diseases. *In*: Kaneko JJ, Harvey JW, Bruss ML (eds): Clinical Biochemistry of Domestic Animals, 5th ed. San Diego, Academic Press, pp. 45–81.

Kaneko JJ: 1997. Serum Proteins and the Dysproteinemias. *In*: Kaneko JJ, Harvey JW, Bruss ML (eds): Clinical Biochemistry of Domestic Animals, 5th ed. San Diego, Academic Press, pp. 117–138.

Kent J: 1992. Acute phase proteins: Their use in veterinary medicine. *Br Vet J* 148:279–282.

Kern MR, Stockham SL, Coates JR: 1992. Analysis of serum protein concentrations after severe thermal injury in a dog. *Vet Clin Pathol* 21:19–22.

Kittleson MD, Johnson LE, Pion PD: 1996. Submaximal exercise testing using lactate threshold and venous oxygen tension as endpoints in normal dogs and in dogs with heart failure. *J Vet Intern Med* 10:21–27.

Kjelgaard-Hansen M: 2009. The use of canine C-reactive protein in clinical practice. *Svensk Veterinartidning* 61:14, 19–24.

Kley S, Caffall Z, Tittle E, et al: 2008. Development of a feline proinsulin immunoradiometric assay and a feline proinsulin enzyme-linked immunosorbent assay (ELISA): A novel application to examine beta cell function in cats. *Dom Anim Endocrinol* 34:311–318.

Kluger EK, Hardman C, Govendir M, et al: 2009. Triglyceride response following an oral fat tolerance test in Burmese cats, other pedigree cats and domestic crossbred cats. *J Fel Med Surg* 11:82–90.

Free ebooks ==> www.Ebook777.com

Klusek J, Kolataj A, Swiderska-Kolacz G: 1997. The influence of starvation on the level of some lipids in pigs (short communication). *Archiv Tierzucht* 40:365–369.

Kohn CW, Knight D, Hueston W, et al: 1989. Colostral and serum IgG, IgA, and IgM concentrations in standardbred mares and their foals at parturition. *J Am Vet Med Assoc* 195:64–68.

Kothe R, Mischke R: 2009. Fructosamine concentration in blood plasma of psittacides—relevance for diagnostics of diabetes? *Praktishe Tierarzt* 90(2):102–109.

Kraft W, Weskamp M, Dietl A: 1994. Untersuchungen zum serum-cholesterin des hundes. *Tierärztl Prax* 22:392–397.

LaVecchio D, Marin LM, Baumwart R, et al: 2009. Serum cardiac troponin I concentration in retired racing greyhounds. *J Vet Intern Med* 23:87–90.

Larsen T, Moller G, Bellio R: 2001. Evaluation of clinical and clinical chemical parameters in periparturient cows. *J Dairy Sci* 84:1749–1758.

Link KR, Rand JS: 2008. Changes in blood glucose concentration are associated with relatively rapid changes in circulating fructosamine concentrations in cats. *J Feline Med Surg* 10:583–592.

Link KR, Rand JS: 1998. Reference values for glucose tolerance and glucose tolerance status in cats. *J Am Vet Med Assoc* 213:492–496.

Linklater AKJ, Lichtenberger MK, Tham DH, et al: 2007. Serum concentrations of cardiac troponin I and cardiac troponin T in dogs with class IV congestive heart failure due to mitral valve disease. *J Veterinary Emergency and Critical Care* 17:243–249.

Lipperheide C, Gothe C, Petersen B, et al: 1997. Haptoglobin quantifications in sera of cattle, pigs and horses with the Nephelometer 100. *Tieraerztliche Umschau* 52:420–422, 425–426.

Lobetti R, Dvir E, Pearson J: 2002. Cardiac troponins in canine babesiosis. *J Vet Intern Med* 16:63–68.

Lumeij JT: 1997. Avian Clinical Biochemistry. *In*: Kaneko JJ, Harvey JW, Bruss ML (eds): Clinical Biochemistry of Domestic Animals, 5th ed. San Diego, Academic Press, pp. 857–883.

Maldonado EN, Casanave EB, Aveldao MI: 2002. Major plasma lipids and fatty acids in four HDL mammals. *Comp Biochem Physiol A Mol Integr Physiol* 132:297–303.

Mansfield CS, James FE, Robertson ID: 2008. Development of a clinical severity index for dogs with acute pancreatitis. *J Am Vet Med Assoc* 233:936–44.

Martinez-Subiela S, Ginel PJ, Ceron JJ: 2004. Effects of different glucocorticoid treatments on serum acute phase proteins in dogs. *Vet Rec* 154:814–7.

McGrotty YL, Knottenbelt CM, Ramsey IK, et al: 2003. Haptoglobin concentrations in a canine hospital population. *Vet Rec* 152:562–564.

McGuire TC: 1988. Failure of colostral immunoglobulin transfer to calves: Prevalence and diagnosis. *Compend Contin Edu* 4:S35-S39.

Mellanby RJ, Herrtage ME: 2002. Insulinoma in a normoglycaemic dog with low serum fructosamine. *J Small Anim Pract* 43:506–508.

Merlo A, Rezende BC, Franchini ML, et al: 2008. Serum amyloid A is not a marker for relapse of multicentric lymphoma in dogs. *Vet Clin Pathol* 37:79–85.

Merlo A, Rezende BC, Franchini ML, et al: 2007. Serum C-reactive protein concentrations in dogs with multicentric lymphoma undergoing chemotherapy. *J Am Vet Med Assoc* 230:522–6.

www.Ebook777.com

Mogg TD, Palmer JE: 1995. Hyperlipidemia, hyperlipemia, and hepatic lipidosis in American miniature horses: 23 cases (1990–1994). *J Am Vet Med Assoc* 207:604–607.

Moore LE, Garvey MS: 1996. The effect of hetastarch on serum colloid oncotic pressure in hypoalbuminemic dogs. *J Vet Intern Med* 10:300–303.

Mori A, Lee P, Mizutani H, et al: 2009. Serum glycated albumin as a glycemic control marker in diabetic cats. *J Vet Diagn Invest* 21:112–116.

Murata H, Shimada N, Yoshioka M: 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J* 168:28–40.

Nakamura M, Takahashi M, Ohno K, et al: 2008. C-reactive protein concentration in dogs with various diseases. *J Vet Med Sci* 70:127–31.

Nazifi S, Dadras H, Hoseinian SA, et al: 2010. Measuring acute phase proteins (haptoglobin, ceruloplasmin, serum amyloid A, and fibrinogen) in healthy and infectious bursal disease virus-infected chicks. *Comp Clin Pathol* 19:283–286.

Nicholas F, Sojka JE, Latour MA, et al: 1999. Effect of hypothyroidism on blood lipid concentrations in horses. *Am J Vet Res* 60:730–733.

Nielsen L, Toft N, Edkersall PD, et al: 2007. Serum C-reactive protein concentration as an indicator of remission status in dogs with muticentric lymphoma. *J Vet Intern Med* 21:1231–6.

Nunokawa Y, Fujinaga T, Taira T, et al: 1993. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci* 55:1011–1016.

Orme CE, Harris RC, Marlin DJ, et al: 1997. Metabolic adaptation to fat-supplemented diet by the thoroughbred horse. *Br J Nutr* 78:443–458.

Oyama MA, Sisson DD: 2004. Cardiac troponin I concentration in dogs with cardiac disease. *J Vet Intern Med* 18:831–9.

Paltrinieri S: 2007. Early biomarkers of inflammation in dogs and cats: the acute phase proteins. *Vet. Res. Communications* 31(Suppl. 1):125–129.

Paltrinieri S: 2008. The feline acute phase reaction. *Vet J* 177:26–35.

Paltrinieri S, Giordano A, Tranquillo V, et al: 2007. Critical assessment of the diagnostic value of feline α_1-acid glycoprotein for feline infectious peritonitis using the likelihood ratios approach. *J Vet Diagn Invest* 19:266–272.

Paltrinieri S, Metzger C, Battilani M, et al: 2007. Serum α_1-acid glycoprotein concentration in non-symptomatic cats with feline coronavirus (FCoV) infection. *Journal of Feline Medicine and Surgery* 9:271–277.

Parra MD, Fuentes P, Tecles F, et al: 2006. Porcine acute phase protein concentrations in different diseases in field conditions. *J Vet Med* 53:488–493.

Parraga ME, Carlson GP, Thurmond M: 1995. Serum protein concentrations in horses with severe liver disease: A retrospective study and review of the literature. *J Vet Intern Med* 9:154–161.

Pazak HE, Bartges JW, Cornelius LC, et al: 1998. Characterization of serum lipoprotein profiles of healthy, adult cats and idiopathic feline hepatic lipidosis patients. *J Nutr* 128:2747S-2750S.

Petersen HH, Nielsen JP, Heegaard PMH: 2004. Applications of acute phase protein measurements in veterinary clinical chemistry. *Vet Res* 35:163–187.

Free ebooks ==> www.Ebook777.com

Prosek R, Sisson DD, Oyama MA, et al: 2007. Distinguishing cardiac and noncardiac dyspnea in 48 dogs using plasma atrial natriuretic factor, B-type natriuretic factor, endothelin, and cardiac troponin I. *J Vet Intern Med* 21:238–42.

Radin MJ, Sharkey LC, Holycross BJ: 2009. Adipokines: A review of biological and analytical principles and an update in dogs, cats, and horses. *Vet Clin Pathol* 38:136–156.

Rath N: 2003. Acute phase proteins in relation to disease and immunity: An avian paradigm [abstract]. Immunol Res Workshop 42–43.

Rath NC, Anthony NB, Kannan L, et al: 2009. Serum ovotransferrin as a biomarker of inflammatory diseases in chickens. *Poultry Science* 88:2069–74.

Rayssiguier Y, Mazur A, Gueux E, et al: 1988. Plasma lipoproteins and fatty liver in dairy cows. *Res Vet Sci* 45:389–393.

Reusch CE, Haberer B: 2001. Evaluation of fructosamine in dogs and cats with hypo- or hyperproteinaemia, azotaemia, hyperlipidaemia and hyperbilirubinaemia. *Vet Rec* 148:370–376.

Reusch CE, Liehs MR, Hoyer M, et al: 1993. Fructosamine: A new parameter for diagnosis and metabolic control in diabetic dogs and cats. *J Vet Intern Med* 7:177–182.

Rishniw M, Barr SC, Simpson KW, et al: 2004. Cloning and sequencing of the canine and feline cardiac troponin I genes. *Am J Vet Res* 65:53–58.

Roman Y, Levrier J, Ordonneau D, et al: 2009. Location of the fibrinogen and albumin fractions in plasma protein electrophoresis agarose gels of five taxonomically distinct bird species. *Revue de Medicine Veterinaire* 160:160–165.

Rogers KS, Forrester SD: 2000. Monoclonal gammopathy. *In:* Feldman BF, Zinkl JG, Jain NC (eds): Schalm's Veterinary Hematology, 5th ed. Philadelphia, Lippincott Williams and Wilkins, pp. 932–936.

Rudloff E, Kirby R: 2000. Colloid osmometry. *Clin Tech Small Anim Pract* 15:119–125.

Sagawa MM, Nakadomo F, Honjoh T, et al: 2002. Correlation between plasma leptin concentration and body fat contents in dogs. *Am J Vet Res* 63:7–10.

Saini PK, Webert DW: 1991. Application of acute phase reactants during antemortem and postmortem meat inspection. *J Am Vet Med Assoc* 198:1898–1901.

Sako T, Mori A, Lee P, Takahashi T, et al: 2008. Diagnostic significance of serum glycated albumin in diabetic dogs. *J Vet Diagn Invest* 20:634–638.

Schmidt O, Deegen E, Fuhrmann H, et al: 2001. Effects of fat feeding and energy level on plasma metabolites and hormones in Shetland ponies. *J Veterinar Med* 48:39–49.

Schober KE, Cornand C, Kirbach B, et al: 2002. Serum cardiac troponin I and cardiac troponin T concentrations in dogs with gastric dilatation-volvulus. *J Am Vet Med Assoc* 221:381–388.

Schulman ML, Nurton JP, Guthrie AJ: 2001. Use of the Accusport semi-automated analyser to determine blood lactate as an aid in the clinical assessment of horses with colic. *J South Afr Vet Assoc* 72:12–17.

Selting KA, Ogilvie GK, Lana SE, et al: 2008. Serum alpha 1-acid glycoprotein concentrations in healthy and tumor-bearing cats. *J Vet Intern Med* 14:503–506.

Shaw SP, Rozanski EA, Rush JE: 2004. Cardiac troponins I and T in dogs with pericardial effusion. *J Vet Intern Med* 18:322–4.

www.Ebook777.com

Sisson D: 2004. Neuroendocrine evaluation of cardiovascular disease. *Vet Clin North Am Sm An Pract* 34:1105–1126.

Solter PF, Hoffman WE, Hungerford LC, et al: 1991. Haptoglobin and ceruloplasmin as determinants of inflammation in dogs. *Am J Vet Res* 52:1738–1742.

Spratt DP, Mellanby RJ, Drury N: 2005. Cardiac troponin I: Evaluation of a biomarker for the diagnosis of heart disease in the dog. *J Small Anim Pract* 46(3):113–114.

Stockham, SL: 1995. Interpretation of equine serum biochemical profile results. *Vet Clinics N Am: Equine Pract* 11:391–414.

Stoddart ME, Whicher JT, Harbour DA: 1988. Cats inoculated with feline infectious peritonitis virus exhibit a biphasic acute phase plasma protein response. *Vet Rec* 123:622–624.

Stokol T, Tarrant JM, Scarlett JM: 2001. Overestimation of canine albumin concentration with the bromcresol green method in heparinized plasma samples. *Vet Clin Pathol* 30:170–176.

Syring RS, Otto CM, Drobatz KJ: 2001. Hyperglycemia in dogs and cats with head trauma: 122 cases (1997–1999). *J Am Vet Med* 218:1124–1129.

Tamamoto T, Ohno K, Ohmi A, et al: 2009. Time-course monitoring of serum amyloid A in a cat with pancreatitits. *Vet Clin Pathol* 38:83–86.

Thomas LA, Brown SA: 1992. Relationship between colloid osmotic pressure and plasma protein concentration in cattle, horses, dogs, and cats. *Am J Vet Res* 53:2241–2244.

Thoresen SI, Aleksandersen M, Lonaas L, et al: 1995. Pancreatic insulin-secreting carcinoma in a dog: Fructosamine for determining persistent hypoglycaemia. *J Small Anim Pract* 36:282–286.

Thoresen SI, Bredal WP: 1999. Serum fructosamine measurement: A new diagnostic approach to renal glucosuria in dogs. *Res Vet Sci* 67:267–271.

Throop JL, Kerl ME, Cohn LA: 2004. Albumin in health and disease: Protein metabolism and function. *Compend Contin Edu* 26:932–936.

van den Berg A, Danuser J, Frey J, et al: 2007. Evaluation of the acute phase protein haptoglobin as an indicator of herd health in slaughter pigs. *Animal Welfare* 16:157–159.

van den Broek AH: 1990. Serum electrophoresis in canine parvovirus enteritis. *Br Vet J* 146:255–259.

van der Kolk JH, Wensing T, Kalsbeek HC, et al: 1995. Lipid metabolism in horses with hyperadrenocorticism. *J Am Vet Med Assoc* 206:1010–1012.

Vandenplatz ML, Moore JN, Barton MH, et al: 2005. Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic. *Am J Vet Res* 66(9):1509–1515.

Vannucchi, CI, Mirandola RM, Oliveira CM: 2002. Acute-phase protein profile during gestation and diestrous: Proposal for an early pregnancy test in bitches. *Animal Reproduction Science* 74(1–2):87–99.

Vasquez-Anon M, Bertics S, Luck M, et al: 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. *J Dairy Sci* 77:1521–1528.

Velga AP, Price CA, deOliveira ST, Dos Santos AP, Campos R, Barbosa PR, Gonzales FH: 2008. Association of canine obesity with reduced serum levels of C-reactive protein. *J Vet Diagn Invest* 20:224–8.

Venkatraman G, Harada K, Gomes AV, et al: 2003. Different functional properties of troponin T mutants that cause dilated cardiomyopathy. *J Biol Chem* 278:41670–41676.

Free ebooks ==> www.Ebook777.com

Vitic J, Tosic L, Stevanovic J: 1994. Comparative studies of the serum lipoproteins and lipids in domestic swine and wild boar. *Acta Veterinaria* 44:49–55.

Watson P, Simpson KW, Bedford PGC: 1993. Hypercholesterolaemia in briards in the United Kingdom. *Res Vet Sci* 54:80–85.

Watson TDG, Barrie J: 1993. Lipoprotein metabolism and hyperlipidaemia in the dog and cat: A review. *J Small Anim Pract* 34:479–487.

Watson TDG, Gaffney D, Mooney CT, et al: 1992. Inherited hyperchylomicronaemia in the cat: Lipoprotein lipase function and gene structure. *J Small Anim Pract* 33:207–212.

Watson TDG, Love S: 1994. Equine hyperlipidemia. *Compend Contin Educ Pract Vet* 16:89–98.

Watson TDG, Packard CJ, Shepherd J: 1993. Plasma lipid transport in the horse (*equus caballus*). *Comp Biochem Physiol* 106B:27–34.

Wiedmeyer CE, DeClue AE: 2008. Continuous glucose monitoring in dogs and cats. *J Vet Intern Med* 22:2–8.

Wiedmeyer CE, Solter PF: 1996. Validation of human haptoglobin immunoturbidimetric assay for detection of haptoglobin in equine and canine serum and plasma. *Vet Clin Pathol* 25:141–146.

Weiser MG, Thrall MA, Fulton R, et al: 1991. Granular lymphocytosis and hyperproteinemia in dogs with chronic ehrlichiosis. *J Am Anim Hosp Assoc* 27:84–88.

Wess G, Reusch C: 2000. Assessment of five portable blood glucose meters for use in cats. *Am J Vet Res* 61: 1587–92.

Wess G, Reusch C: 2000. Evaluation of five portable blood glucose meters for use in dogs. *J Am Vet Med Assoc* 216:203–209.

Whitney MS: 1992. Evaluation of hyperlipidemias in dogs and cats. *Semin Vet Med Surg (Small Animal)* 7:292–300.

Xie H, Newberry L, Clark FD, et al: 2002. Changes in serum ovotransferrin levels in chickens with experimentally induced inflammation and diseases. *Avian Diseases* 46:122–131.

Yilmaz Z, Senturk S: 2007. Characterization of lipid profiles in dogs with parvoviral enteritis. *J Small Anim Pract* 48(11):643–650.

Zvorc A, Matijatko V, Beer B, et al: 2000. Blood serum proteinograms in pregnant and non-pregnant cows. *Veterinarski Archiv* 70:21–30.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 7

LIVER

Perry J. Bain, DVM, PhD

HEPATIC ABNORMALITIES DETECTED BY LABORATORY TESTS

Serum chemistry can be used to detect several types of hepatic abnormalities. These include injury or necrosis to the hepatocytes, alterations in the synthetic or excretory functions of the liver, cholestasis, and altered portal circulation.

I. HEPATOCELLULAR LEAKAGE/NECROSIS

A. Altered permeability of hepatocyte cell membranes results in leakage of cytosolic enzymes into extracellular fluid and, subsequently, into the blood.

B. Leakage may occur with either sublethal (reversible) hepatocellular injury or hepatocellular necrosis.

C. The magnitude of the increase in serum enzymatic activity depends on the number of hepatocytes affected, the severity of the injury, and the serum half-life of the enzyme.

D. Following hepatocellular injury, increased serum enzyme activity is evident within hours.

E. Hepatocellular leakage enzyme activity is a more sensitive indicator of hepatic damage than tests of liver function.

F. Hepatocellular injury often is accompanied by cellular swelling, inflammation, and/or necrosis, which may alter bile flow, resulting in concurrent intrahepatic cholestasis (and increases in "cholestatic" parameters).

G. Commonly measured enzymes with increased serum activity resulting from hepatocellular leakage include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sorbitol dehydrogenase (SDH). These enzymes vary in their concentration in liver tissue and specificity for liver disease in different species.

II. DECREASED HEPATIC FUNCTIONAL MASS (HEPATIC INSUFFICIENCY)

A. A sufficient number of functional hepatocytes must be present for normal liver functions to occur. Approximately 70% or more of the functional hepatocytes must be lost before alterations of hepatic function are detectable by serum biochemical testing.

B. Mechanisms of decreased hepatic functional mass

1. Hepatocellular injury or necrosis
2. Hepatocellular loss in chronic liver disease with replacement by fibrous connective tissue (cirrhosis)
3. Hepatic atrophy (often associated with chronic portosystemic shunts)

www.Ebook777.com

C. Hepatic functions that are commonly evaluated by laboratory tests include:
1. Protein synthesis (albumin, α- and β-globulins, clotting factors)
2. Uptake and excretion of bilirubin and bile acids
3. Uptake of ammonia and conversion of ammonia to urea (BUN)
4. Glucose homeostasis and glycogen storage
5. Removal o f enteric antigens and endogenous antibodies by Kupffer cells (fixed macrophages) in the hepatic sinusoids
6. Uptake and excretion of exogenous dyes (e.g., bromosulphalein [BSP, sulfobromophthalein] and indocyanine green [ICG])

III. CHOLESTASIS

A. Cholestasis is the interruption or obstruction of bile flow or excretion.
1. Intrahepatic cholestasis occurs within the bile canaliculi and bile ductules of the liver.
2. Extrahepatic cholestasis occurs outside of the liver in the gallbladder or common bile duct.
3. Cholestasis may result from physical obstruction of bile flow (e.g., inflammation, infection, cholelithiasis, neoplasia) or from metabolic derangements (e.g., hepatotoxicity, fasting horses, sepsis, or hereditary defects in bile secretion).

B. Cholestasis may cause induction and release of certain membrane-bound hepatic enzymes, such as alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT), increasing their serum activities.

C. Cholestasis also results in retention or reflux of bile, increasing serum concentrations of substances that are normally excreted in bile (bilirubin, bile acids).

D. Cholestasis also may cause hepatocellular damage due to the retention of bile acids, which have a detergent action on cellular membranes.

IV. ALTERATION OF PORTAL BLOOD FLOW

A. The blood flow of the liver is unique.
1. The hepatic artery provides oxygenated blood from the systemic circulation.
2. The portal vein provides blood flow from the intestinal tract and spleen.
3. The hepatic vein returns blood from the liver to the systemic circulation.

B. Portal blood constituents are absorbed from the gastrointestinal tract.
1. Bile acids, amino acids, glucose, ammonia, medium-length fatty acids, and intestinal antigens are absorbed via the portal blood.
2. These substances are largely removed from the blood by the liver before they reach the systemic circulation.
3. Acquired or congenital portosystemic shunts bypass the liver and deliver portal blood directly into the systemic circulation. When this occurs, systemic blood concentrations of substances that are normally removed by hepatic processing (e.g., bile acids, ammonia) are increased.
4. Chronic portosystemic shunts may result in hepatic atrophy due to decreased concentrations of intestinal and pancreatic hepatotrophic factors delivered to the liver.

HEPATIC LABORATORY TESTS

Alterations in serum chemistry parameters often are useful in detecting hepatic disease. These abnormalities may include altered activities of hepatic enzymes due to hepatocellular damage or enzyme induction, increased concentration of substances normally removed or excreted by the liver, or

Free ebooks ==> www.Ebook777.com

alterations in the concentrations of substances produced by hepatic synthesis. These serum biochemical alterations may not be specific for hepatic disease and may not indicate a specific cause of hepatic dysfunction. For example, hyperbilirubinemia may be due to hemolytic disease, decreased hepatic functional mass, or cholestasis. Increased serum bile acid concentrations may result from loss of hepatic functional mass, portosystemic shunts, or cholestasis.

I. HEPATIC ENZYME ALTERATIONS

Alterations in serum hepatic enzyme activity provide a sensitive means to evaluate liver disease. Changes in enzyme activity are often detected before hepatic failure is identified. Hepatic enzymes can be broadly divided into two categories: hepatocellular leakage enzymes and induced (or cholestatic) enzymes. Species vary in the activity of enzymes expressed in hepatic tissue, as well as in their enzymatic responses to induction.

A. Hepatocellular leakage enzymes

1. General characteristics

a. Hepatocellular leakage enzymes are soluble cytosolic enzymes that have a high activity in hepatocytes (and often in other tissues).

b. They are released with damage to the hepatocyte membranes in association with sublethal injury or hepatocellular necrosis.

c. The serum enzyme activity of hepatocellular leakage enzymes depends upon the number of hepatocytes injured, the severity of the injury, and the half-life of the enzyme.

d. The magnitude of the increase in enzymatic activity does not necessarily correlate with clinical manifestations of hepatic insufficiency.

e. In chronic, progressive liver diseases, a low proportion of hepatocytes may be undergoing damage or necrosis at any particular time. Therefore, activity of hepatocellular leakage enzymes may be within the reference interval or only mildly increased, despite the presence of hepatic insufficiency.

f. Hepatic atrophy may result in decreased serum enzyme activity due to lower numbers/mass of hepatocytes.

g. In some cases, increased serum activity of these enzymes originates in other tissues such as skeletal or cardiac muscle, or from erythrocytes (*in vitro* or intravenous hemolysis). Concurrent measurement of creatine kinase activity, a specific indicator of muscle injury, may distinguish muscle injury from hepatic disease as a potential cause of increased leakage enzyme activity in serum.

h. Commonly evaluated cytoplasmic enzymes include ALT, AST, SDH, LDH, and GDH.

2. Alanine aminotransferase (ALT)

a. The obsolete name for this enzyme is serum glutamic pyruvic transaminase (SGPT).

b. Increased serum ALT activity occurs with sublethal hepatocellular injury or necrosis in the dog and cat.

c. Muscle necrosis also may cause increased serum activity of ALT.

d. Liver ALT activity is very low in horses, ruminants, pigs, and birds. Increases in serum ALT activity in these species are more likely the result of muscle injury. Therefore, ALT is often not tested in large animal chemistry panels.

e. Mild to moderate increases in ALT activity may occur with anticonvulsants, corticosteroids (dog), and thiacetarsemide. This is most likely due to mild hepatocellular injury rather than induction.

f. Trauma (e.g., automobile injuries) may cause dramatic increases in ALT activity, probably from a combination of liver injury and muscle damage. Blood loss or shock associated with trauma also may be associated with increased ALT activity from centrilobular necrosis secondary to ischemia.

g. ALT has a clearance half-life of approximately 60 hours in the dog.

www.Ebook777.com

h. Following a single toxic insult, ALT activity increases within 12 hours, peaks within one to two days, and returns to the reference interval over two to three weeks.

3. Aspartate aminotransferase (AST)

a. The obsolete name for this enzyme is serum glutamic oxaloacetic transaminase (SGOT).

b. Both cytosolic and mitochondrial isoenzymes of AST exist. More severe cellular injury is required for release of the mitochondrial isoenzyme.

c. AST is not liver specific; it is found in most tissues. Significant increases in serum AST activity may occur with liver or muscle injury or with hemolysis.

d. Muscle necrosis should be excluded by assessment of creatine kinase activity before concluding that increased AST activity is the result of liver disease.

e. Erythrocytes contain AST; thus, increased serum AST activity may occur with *in vivo* or *in vitro* hemolysis. Covert hemolysis, which occurs when the serum is left on the clot, may result in increased AST activity even though visible hemolysis is not apparent.

f. AST usually has a shorter half-life in serum than ALT, with a clearance half-life of approximately 12 hours in the dog.

4. Sorbitol dehydrogenase (SDH)

a. The liver of all animals contains high SDH activity. Increases in serum SDH activity generally are considered liver specific in all species studied.

b. SDH is the enzyme of choice to detect hepatocellular injury in horses, sheep, goats, and cattle, because it is more specific for hepatic disease than AST and ALT in these species.

(1) AST is not a liver-specific enzyme.

(2) Hepatic ALT activity is too low to serve as a marker of hepatocellular injury in these species.

c. Serum activity of SDH has a short clearance half-life (less than 12 hours); SDH activity may return to baseline values within a few days after a single hepatocellular insult.

d. SDH is labile *in vitro*.

(1) Serum samples for SDH assays should be frozen if not tested immediately.

(2) Serum samples for SDH testing should not be mailed to the laboratory at ambient temperature.

5. Lactate dehydrogenase (LDH)

a. High lactate dehydrogenase activity is found in many types of cells and should not be considered liver specific.

b. Increased LDH activity may result from hemolysis, muscle damage, or hepatocellular injury (similar to AST).

6. Glutamate dehydrogenase (GDH, GLDH) (birds)

a. GDH is a cytosolic enzyme, and increases with hepatic necrosis.

b. This enzyme is considered to be liver specific in birds but has low sensitivity.

B. Induced hepatic enzymes

1. General characteristics

a. These enzymes are typically membrane-bound and are not released into the serum with increased membrane permeability.

b. Increased serum enzymatic activity is the result of enzyme induction, typically as a result of cholestasis, drug, or hormonal effects.

2. Alkaline phosphatase (ALP)

a. In the hepatocytes and biliary epithelial cells, ALP is predominantly bound to the plasma membrane.

b. ALP is less useful for the detection of cholestasis in large animals (cattle, horse, sheep, goats).

(1) ALP activity has wide reference intervals in these species. Therefore, it is not a sensitive indicator of biliary disease.

(2) GGT is the preferred indicator of cholestasis for these animals.

Free ebooks ==> www.Ebook777.com

c. Increased serum ALP activity may occur in:
- **(1)** Cholestatic diseases
- **(2)** Bone lysis or remodeling (e.g., bone tumors, young, growing animals)
- **(3)** Corticosteroid treatment or Cushing's disease (dogs)
- **(4)** Phenobarbital treatment (likely due to hepatic injury/cholestasis)
- **(5)** Hepatic nodular hyperplasia (dogs)
- **(6)** Colic in horses
- **(7)** Feline hepatic lipidosis
- **(8)** Hyperthyroidism in cats

d. Isoenzymes of ALP are found in different tissues, and serum activity of these isoenzymes varies depending on the cause.
- **(1)** The various glycosylation forms of the enzymes derived from the two ALP genes (intestinal and tissue nonspecific) are commonly referred to as isoenzymes.
- **(2)** Total serum ALP activity is typically measured in serum chemistry panels, but isoenzymes may be quantified by electrophoresis or by selective suppression of isoenzymes.
- **(3)** Clinically important ALP isoenzymes include the hepatic, bone, intestinal, and corticosteroid- induced forms. The corticosteroid isoenzyme of ALP has been identified only in dogs.

e. ALP isoenzymes can be differentiated by their electrophoretic mobility or by chemical- or heat-induced suppression of enzymatic activity *in vitro*.
- **(1)** Liver ALP isoenzyme (LALP)
  - ***(a)*** Intrahepatic or extrahepatic cholestasis causes a marked increase in ALP activity, which is predominately the hepatic isoenzyme.
  - ***(b)*** Increased ALP activity is a sensitive indicator of cholestasis and precedes the development of hyperbilirubinemia. Focal cholestatic lesions may increase ALP activity without causing hyperbilirubinemia.
  - ***(c)*** Cats have lower hepatic ALP activity than dogs. Feline ALP also has a shorter half-life than canine ALP.
  - ***(d)*** In cats with cholestatic disease, increases in ALP activity are usually less dramatic than increases in GGT activity. Feline hepatic lipidosis is an exception; ALP activity typically increases to a greater extent than GGT activity.
  - ***(e)*** Increased ALP activity is observed commonly in hyperthyroid cats. Increased ALP enzymatic activity may consist of both liver and bone isoenzymes.
  - ***(f)*** The hepatic ALP isoenzyme has a serum clearance half-life of approximately 72 hours in the dog.
- **(2)** Corticosteroid ALP isoenzyme (CALP)
  - ***(a)*** Endogenous or exogenous glucocorticoids may cause a marked induction of ALP activity (up to 200× normal activity) in the dog.
  - ***(b)*** The initial increase in serum ALP activity following corticosteroid treatment is in the hepatic isoenzyme.
  - ***(c)*** Following corticosteroid treatment, the corticosteroid isoenzyme (an alternate glycosylation form of intestinal ALP, produced in the liver) increases gradually, becoming the predominant form of ALP in serum over several weeks.
  - ***(d)*** In hyperadrenocorticism, the corticosteroid isoenzyme often is the predominant form of ALP by the time the disease is detected.
  - ***(e)*** Increased serum ALP activity may persist for weeks to months after glucocorticoid treatment is discontinued.
  - ***(f)*** Levamisole inhibition or heat inactivation in vitro can be used to distinguish some of the isoenzymes of ALP.
    - *i)* Activity of the corticosteroid and intestinal isoenzymes of ALP is largely resistant to levamisole inhibition or heat inactivation.

www.Ebook777.com

ii) Activity of the hepatic and bone isoenzymes of ALP is markedly decreased or eliminated by levamisole inhibition or heat inactivation.

iii) Increased ALP activity following levamisole or heat treatment of the sample can be considered evidence of increased corticosteroid ALP.

(g) Corticosteroid ALP activity also may be induced by drug treatment (including anticonvulsants), some hepatic neoplasms, or chronic diseases such as diabetes mellitus.

(h) The corticosteroid ALP isoenzyme has a serum clearance half-life of approximately 72 hours in the dog.

(3) Bone ALP isoenzyme (BALP)

(a) Increased activity (up to 3× adult values) of the bone isoenzyme of ALP may be observed in young, rapidly growing animals, animals with lytic or proliferative bone lesions (e.g., osteosarcoma, plasma cell myeloma), or animals with active bone resorption (e.g., primary or secondary hyperparathyroidism).

(b) A benign familial hyperphosphatasemia has been described in Siberian Huskies. These dogs have markedly increased ALP activity (predominantly of the bone isoenzyme) with no other evidence of hepatic disease.

(c) High mean ALP activity has been found in Scottish Terriers (greater than 1,500 U/L) without evidence of liver disease or history of corticosteroid treatment.

(d) The bone and liver isoenzymes of ALP may be differentiated in vitro by precipitation of the bone ALP isoenzyme with wheat germ lectin. Differentiation of these isoenzymes is usually a research technique.

(e) In dogs with osteosarcoma, increased serum alkaline phosphatase levels are associated with shorter survival times.

(f) The bone ALP isoenzyme has a serum clearance half-life of approximately 72 hours in the dog.

(4) Intestinal and placental ALP isoenzyme (IALP)

(a) In the dog and cat, the intestinal isoenzyme of ALP constitutes only a small fraction of total serum ALP activity. Although the intestine of dogs and cats contains considerable ALP activity, this isoenzyme has a short half-life in serum (less than six minutes) and does not contribute significantly to serum ALP activity.

(b) The placental isoenzyme of ALP may be increased in late-term pregnancy in animals. The plasma clearance half life of this isoenzyme is less than six minutes in the dog.

(c) Serum ALP may be increased in horses with colic due to increased intestinal isoenzyme activity. Increased ALP activity also may be found in the abdominal fluid of horses with colic.

3. Gamma glutamyl transferase (GGT or γGT)

a. GGT, like ALP, is predominantly associated with the brush border or microvilli of hepatocytes, biliary epithelial cells, renal tubular epithelial cells, and mammary epithelial cells (especially during lactation).

b. Increased GGT activity in serum is the result of enzyme induction involving hepatocytes or biliary epithelial cells.

c. Cholestasis causes increased serum GGT activity.

d. GGT is considered more sensitive for cholestasis (compared to ALP) in cats, horses, and cattle.

e. GGT also may be increased with severe hepatic necrosis in horses and cattle, presumably due to necrosis of biliary epithelium.

f. Serum activity of GGT may be induced by corticosteroid administration in dogs.

g. Colostrum of dogs, sheep, and cattle contains high GGT activity.

(1) Neonates may have very high serum GGT activity (up to 1,000× adult activity).

(2) GGT may be useful as an indicator of passive transfer in these species.

(3) The lactating mammary epithelium is the source of the increased enzymatic activity.

h. GGT is the preferred indicator of biliary disease in birds.

i. GGT has a serum clearance half-life of approximately three days in the dog.

Free ebooks ==> www.Ebook777.com

j. Increased GGT activity may be detected in urine with renal tubular injury. Increases in the urinary GGT:creatinine ratio can be used as an early indicator of nephrotoxicity.

II. TESTS OF HEPATIC UPTAKE, CONJUGATION, AND SECRETION

A. Bilirubin (Figure 7.1)

1. Metabolism

a. Bilirubin is a pigment that is produced by the degradation of the heme portion of hemoglobin and myoglobin and, to a smaller extent, non-heme porphyrins.

b. Most bilirubin is produced in mononuclear phagocytes.

c. Birds lack biliverdin reductase and do not form significant quantities of bilirubin.

d. Bilirubin is metabolized to urobilinogen by bacteria in the intestine. This can be reabsorbed and excreted in urine. Urine urobilinogen may be increased with increased bilirubin delivery to the GI tract (hemolytic disease) or decreased absent in patients with biliary obstruction or intestinal malabsorption.

2. Types of bilirubin

a. Unconjugated bilirubin

(1) Unconjugated bilirubin is not water soluble.

(2) It is transported in the blood bound to albumin by an ionic bond.

b. Conjugated bilirubin

(1) Unconjugated bilirubin is dissociated from albumin at the hepatocyte cell membrane and taken into the hepatocyte by plasma membrane transporters.

(2) Conjugated bilirubin is formed in the hepatocyte by glucuronidation, which renders the molecule water soluble.

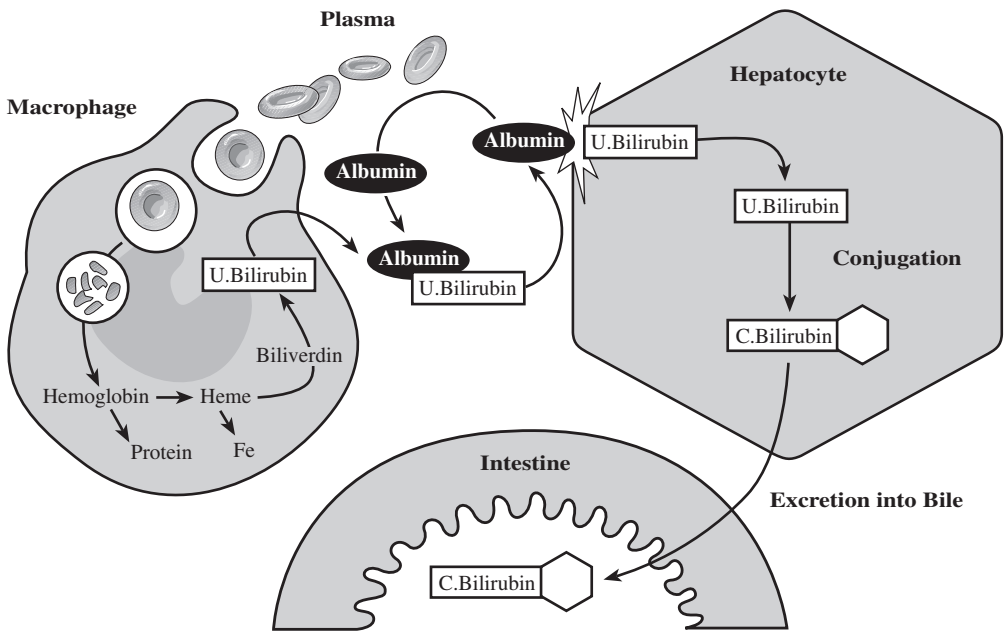


FIGURE 7.1. Bilirubin metabolism. Bilirubin is produced as a breakdown product of hemoglobin. The unconjugated bilirubin (U. Bilirubin) is transported in the blood (ionically bound to albumin) to the liver. Unconjugated bilirubin is taken up by hepatocytes, conjugated (C. Bilirubin), and excreted into bile.

www.Ebook777.com

(3) Conjugated bilirubin is secreted into the bile canaliculi and flows to the intestine via the biliary system.

c. Biliprotein (delta bilirubin)

(1) Biliprotein is conjugated bilirubin that is covalently and irreversibly bound to serum albumin.

(2) Variable quantities of biliprotein may be formed in hyperbilirubinemic conditions.

(3) The primary significance of biliprotein is that it has a long half-life in serum (essentially equivalent to the Zhalf-life of albumin).

(4) High concentrations of biliprotein may account for the prolonged hyperbilirubinemia that may be observed following the resolution of hepatic disease.

3. Bilirubin measurement

a. Bilirubin measurements are based on the diazo reaction.

(1) Conjugated bilirubin reacts directly with this reagent to form an azo dye.

(2) Unconjugated bilirubin reacts with this reagent only after the addition of a solubilizing reagent.

b. Direct-reading bilirubin is the bilirubin concentration without the addition of alcohol. Direct-reading bilirubin is synonymous with conjugated bilirubin.

c. Total bilirubin is quantified following the addition of the solubilizing reagent to the reactants.

d. Indirect-reading bilirubin is the mathematical difference between total bilirubin and the direct-reading bilirubin. Indirect-reading bilirubin is synonymous with unconjugated bilirubin.

4. Hyperbilirubinemia is an increased serum concentration of bilirubin. Hyperbilirubinemia may cause visible discoloration of tissues or body fluids (skin, sclera, gingival, serum, etc.), a condition called icterus or jaundice. Causes of hyperbilirubinemia include the following:

a. Increased bilirubin production (pre-hepatic hyperbilirubinemia).

(1) Increased erythrocyte breakdown with hemolytic disease or following internal hemorrhage causes increased bilirubin production.

(2) The resulting increase in bilirubin concentration overwhelms hepatic uptake, conjugation, and/or secretion capacities.

b. Decreased hepatic uptake or conjugation (hepatic hyperbilirubinemia).

(1) Loss of hepatic function results in decreased capacity for bilirubin uptake and conjugation.

(2) Anorexia or fasting in horses decreases bilirubin uptake by hepatocytes. Total bilirubin concentration may reach 10 mg/dL in otherwise healthy horses.

(3) Sepsis may decrease bilirubin uptake.

c. Cholestasis (post-hepatic hyperbilirubinemia).

(1) Decreased secretion of bilirubin into bile.

(2) Physical obstruction of bile flow may occur intraheptically with neoplastic disease, hepatocyte swelling in hepatic lipidosis, or corticosteroid hepatopathy.

(3) Extrahepatic cholestasis may occur with cholangitis, cholelithiasis, cholecystitis, and pancreatitis.

5. Bilirubinuria

a. Conjugated bilirubin passes through the glomerular filter into the urine.

b. Unconjugated bilirubin and biliprotein are albumin bound. This molecular complex is too large to pass through the normal glomerular filter.

c. Canine renal tubular epithelial cells have a limited ability to conjugate and excrete bilirubin into the urine. Healthy dogs, especially males, therefore often have trace bilirubinuria with concentrated (greater than 1.040) urine.

d. Bilirubinuria normally precedes the development of hyperbilirubinemia, especially in hepatic or post-hepatic causes of hyperbilirubinemia.

e. With marked bilirubinuria, bilirubin crystals may appear in the urine sediment.

6. Conjugated vs. unconjugated bilirubin concentrations in interpretation of hyperbilirubinemia

a. Comparison of relative concentrations of unconjugated and conjugated bilirubin can be used to attempt to characterize the cause of hyperbilirubinemia.

Free ebooks ==> www.Ebook777.com

b. Unconjugated bilirubin should theoretically predominate in pre-hepatic or hepatic hyperbilirubinemia, and conjugated bilirubin should predominate in post-hepatic hyperbilirubinemia.

c. Assessment of conjugated vs. unconjugated bilirubin can be complicated by the following factors:

d. Unconjugated bilirubin is expected to predominate in hemolytic disease (pre-hepatic hyperbilirubinemia). However, if the secretory function of the liver is overwhelmed, increased concentrations of conjugated bilirubin may be observed.

e. Hyperbilirubinemia from any cause in horses and cattle typically consists predominantly of unconjugated bilirubin.

f. Hepatocellular disease may cause increased concentrations of both unconjugated bilirubin and conjugated bilirubin.

(1) The presence of unconjugated bilirubin is due to decreased bilirubin uptake and conjugation by damaged hepatocytes.

(2) The presence of conjugated bilirubin is due to intrahepatic cholestasis from hepatocyte swelling.

g. Post-hepatic biliary obstruction is expected to increase conjugated bilirubin concentration, but hepatic injury secondary to cholestasis also may result in increased concentration of unconjugated bilirubin.

h. Dogs and cats with pre-hepatic or post-hepatic hyperbilirubinemia often have a mixture of conjugated and unconjugated bilirubin.

i. Evaluation of other laboratory parameters may provide more information about the cause of hyperbilirubinemia than does the percentage of conjugated vs. unconjugated bilirubin.

(1) Anemia suggests hemolytic disease, particularly with evidence of a hemolytic anemia (spherocytes, positive Coombs' test, Heinz bodies, etc.).

(2) Increased GGT or ALP activity in conjunction with hyperbilirubinemia suggests cholestatic disease.

B. Biliverdin (birds)

1. Birds lack biliverdin reductase and form biliverdin instead of bilirubin as the primary breakdown product of heme.

2. Biliverdinemia is rare in birds; however, green, bile-stained urates may be observed in the feces during liver disease.

C. Bile acids (Figure 7.2)

1. Bile acids (also known as bile salts) are synthesized in the liver from cholesterol. They are conjugated and secreted into bile, where they function to solubilize lipids and aid in fat digestion in the intestine.

2. Most of the bile acids excreted in bile are resorbed in the portal circulation and recycled. This process is termed enterohepatic circulation. Bile acids are efficiently removed from portal blood by cellular transporters on the sinusoidal membrane of hepatocytes.

3. Bile acids may contribute to hepatocellular membrane damage in cholestasis through a detergent action.

4. Bile acid testing

a. In dogs and cats, baseline (fasting) and postprandial serum bile acid levels are measured. Feeding induces the release of a bolus of bile into the intestine from the gallbladder. The measurement of a two-hour postprandial bile acid concentration increases the sensitivity of the test, providing a more severe test of the liver's capacity for bile acid resorption.

b. Postprandial serum samples are often lipemic, which may falsely elevate bile acid concentration because of interference with light transmission in the spectrophotometric assay. Methods used to remove lipoproteins prior to serum chemistry analysis also may remove bile acids that are bound to the lipoproteins.

www.Ebook777.com

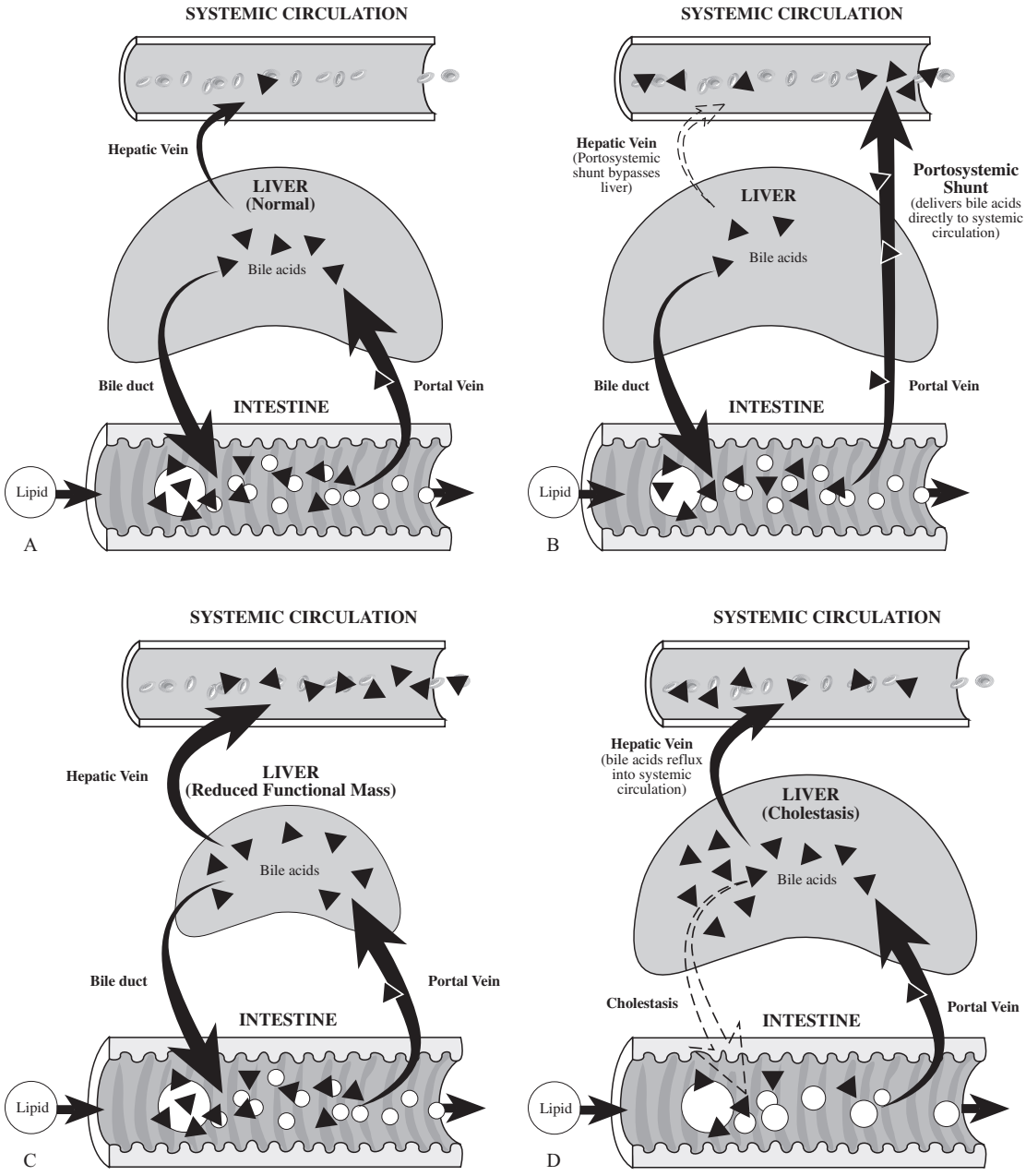


FIGURE 7.2. a. Bile acid metabolism. Bile acids are produced by hepatocytes and delivered via the bile duct to the intestine, where they aid in lipid digestion. Most of the bile acids are subsequently absorbed from the intestine by the portal circulation. In normal animals, most of the portal blood bile acids (approximately 70% to 95%) are removed by hepatocytes and re-excreted into bile (enterohepatic recirculation). b. Bile acid metabolism (continued). Portosystemic shunts bypass the liver and deliver bile acids directly from the portal blood to the systemic circulation. Bile acid removal by hepatocytes from the systemic circulation is less efficient than uptake from the portal circulation, and serum bile acid levels increase. c. Bile acid metabolism (continued). With reduced hepatic functional mass, removal of bile acids from portal blood is less efficient, resulting in increased serum bile acid levels. d. Bile acid metabolism (continued). In cholestatic disease, bile acids reflux into the systemic circulation, increasing serum bile acid levels.

Free ebooks ==> www.Ebook777.com

c. In horses, a single bile acid measurement is taken. Because horses lack a gallbladder, there is no advantage in measuring a postprandial sample.

d. Interpretation of bile acid concentrations in birds is similar to that in mammals, but postprandial responses are more variable.

e. In ruminants, bile acids are a less sensitive indicator of hepatic disease because of wide reference intervals.

f. Bile acids are relatively stable at room temperature.

5. Increased bile acid concentration

a. Portosystemic shunts typically cause increased bile acid concentration.

(1) Portal blood (with its high concentration of bile acids) bypasses the liver and enters the systemic circulation. Bile acid uptake from systemic blood is less efficient than from portal blood.

(2) Hepatic atrophy resulting from a chronic shunt produces a loss of hepatic functional mass, resulting in decreased ability to remove bile acids from blood.

b. In some animals with portosystemic shunts, baseline bile acid concentration may be within the reference interval while postprandial bile acid concentration is increased.

c. In liver failure, a loss of functional hepatic mass results in increased bile acid concentration due to decreased bile acid recycling.

d. Cholestasis causes reflux of bile acids into the bloodstream, increasing bile acid concentrations.

e. Inappropriate contraction of the gallbladder may cause an elevated fasting bile acid concentration.

6. Decreased bile acid concentration

a. Small intestinal disease (ileal malabsorption) may cause decreased bile acid resorption. This may complicate the assessment of concurrent hepatic disease.

b. Although bile acids are synthesized by the liver, decreased bile acid concentration usually is not observed in hepatic failure. This may be explained by the recirculation and reutilization of bile acids, as well as the low reference intervals for serum bile acid values.

D. Ammonia (Figure 7.3)

1. Ammonia is produced in the gastrointestinal tract by the breakdown of amino acids and urea by gastrointestinal microflora.

a. Some ammonia also is produced by amino acid deamination in other body cells.

b. The ammonia produced in the gastrointestinal tract is transported by the portal circulation to the liver.

c. In mammals, ammonia is converted to urea by the hepatic urea cycle enzymes.

d. Birds have higher plasma ammonia concentrations than mammals.

2. Because ammonia is volatile, special handling is required for accurate measurement.

a. Blood samples must be collected in ammonia-free heparin.

b. Plasma should be separated immediately from blood cells.

c. The sample should be chilled on ice and analyzed without delay (preferably within 15 to 30 minutes).

d. If the specimen cannot be analyzed immediately, it should be quick-frozen and analyzed within two days.

e. The lability of the specimen and special handling requirements limit the usefulness of plasma ammonia measurements in most clinical settings.

3. Blood samples for baseline ammonia determination usually are collected after an eight- to 12-hour fast.

4. The sensitivity in detecting impaired ammonia uptake and metabolism may be increased by performing an ammonia tolerance test.

a. This test is contraindicated if baseline hyperammonemia is present because central nervous system disease may occur.

www.Ebook777.com

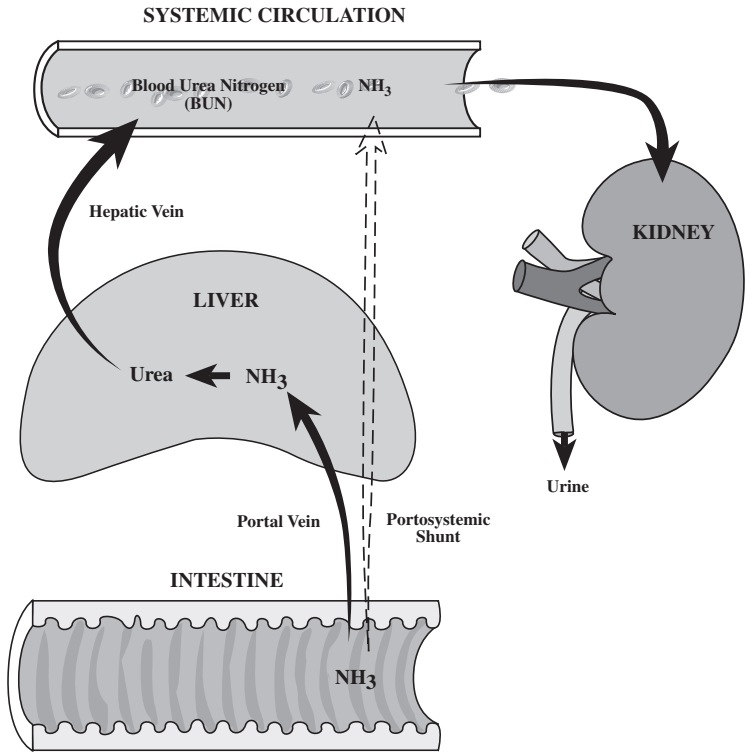


FIGURE 7.3. Ammonia metabolism. Ammonia is produced in the gastrointestinal tract by enteric microflora (as well as amino acid metabolism in cells) and transported to the liver by the portal circulation. Most of the ammonia is converted to urea in the liver. The urea enters the systemic circulation (blood urea nitrogen, BUN) and is excreted in urine. With portosystemic shunting of blood, ammonia bypasses the liver and enters the systemic circulation, resulting in hyperammonemia (dashed arrow).

b. In dogs, 100 mg/kg of ammonium chloride solution (20% w/v, maximum dose 3 g) is given orally or by stomach tube.

c. The plasma ammonia concentration is measured initially (baseline) and 30 minutes after ammonium chloride administration.

d. In animals with adequate hepatic function, post-challenge ammonia concentrations should be equal to the baseline value or increased up to two-fold. Animals with hepatic insufficiency have ammonia concentrations that are increased three- to 10-fold above the baseline ammonia concentration.

5. Hyperammonemia (increased plasma ammonia concentration)

a. Hepatic insufficiency may result in hyperammonemia due to decreased liver uptake and conversion of ammonia to urea.

(1) Decreased blood urea nitrogen (BUN) concentration also may be present due to the decreased conversion of ammonia to urea.

(2) Loss of 70% of hepatic functional mass may be required before baseline hyperammonemia is observed. Ammonia tolerance testing may detect hepatic disease before this degree of loss of function occurs.

b. Portosystemic venous shunting may cause hyperammonemia as portal blood bypasses the liver and enters the systemic circulation. Hepatic atrophy due to the shunt may further contribute to hyperammonemia.

Free ebooks ==> www.Ebook777.com

(1) Animals with congenital portosystemic venous shunts often have baseline ammonia values that are three- to 10-fold above the reference interval.
(2) An animal with a portosystemic shunt occasionally will have a baseline ammonia value within the reference interval. In such cases, an ammonia tolerance test may be required to demonstrate hyperammonemia.

c. Ammonium biurate crystals may be observed in the urine of animals with hyperammonemia.

d. Hyperammonemia may develop in ruminants fed excessive urea or ammoniated feed additives as a non-protein nitrogen source. Urea is broken down to ammonia by ruminal microflora, and the ammonia is absorbed into the systemic circulation.

e. Hyperammonemia due to congenital deficiencies of urea cycle enzymes has been reported in the dog, but is rare.

E. Hepatic photosensitivity

1. Phylloerythrin is a porphyrin that is produced as a breakdown product of chlorophyll by the gastrointestinal microflora of herbivores. It is absorbed via the portal circulation, where it is normally removed by hepatocytes and excreted into bile.
2. In hepatic insufficiency, phylloerythrin enters the systemic circulation and accumulates in the skin.
3. Cutaneous phylloerythrin reacts with sunlight, resulting in free radical formation with subsequent inflammation and necrosis.
4. Affected animals develop dermatitis, usually affecting unhaired and/or unpigmented areas of the skin most severely.

F. Dye clearance tests

1. Bromosulphalein (BSP) and indocyanine green (ICG) are organic dyes that are removed from blood by the liver and excreted into bile.
2. The clearance rate of these dyes following intravenous injection is used as a test of liver function and bile flow.
3. Technical difficulties, limited dye availability, and expense have largely relegated the use of these tests to research studies. These tests have been largely replaced by bile acid testing in clinical diagnostic testing.

III. TESTS AFFECTED BY ALTERATIONS IN HEPATIC SYNTHESIS AND HOMEOSTASIS

The liver is responsible for the synthesis and homeostasis of a wide variety of serum constituents. Loss of hepatic functional mass may cause decreased synthesis and a decline in the concentration of certain analytes. Hepatic homeostasis of certain serum constituents also may be altered with hepatic failure. The values of these analytes may fluctuate widely in disease. Because the liver has a large reserve capacity, these laboratory abnormalities typically are not detectable until approximately 70% of hepatic functional mass has been lost.

A. Glucose (Chapter 6)

1. Abnormalities in glucose homeostasis may occur with reduced hepatic functional mass (70% or greater loss of function).
2. Reduced hepatic glycogen stores and decreased insulin clearance may cause fasting hypoglycemia.
3. Prolonged postprandial hyperglycemia may occur in hepatic insufficiency due to decreased hepatic uptake of glucose from portal blood (hepatic insufficiency and/or portosystemic shunting).

B. Albumin and globulin concentrations and A/G ratio (Chapter 6)

1. Albumin has a relatively long serum half-life (approximately seven to 10 days); therefore, normoalbuminemia is present during acute liver disease.

www.Ebook777.com

2. Hypoalbuminemia may accompany chronic liver failure or atrophy.
   a. Hypoalbuminemia is not a specific indicator of hepatic insufficiency.
   b. Hypoalbuminemia also may occur with starvation, cachexia, malabsorption, inflammation, hemorrhage, proteinuria of glomerular disease, protein-losing enteropathy, severe exudative skin disease, burns, intestinal parasitism, high-protein effusions, or inflammation.
3. The concentrations of some α- and β-globulins also decrease with hepatic insufficiency, but these changes usually have a mild effect on total globulin levels.
4. In chronic liver disease, the hypoalbuminemia is often accompanied by hyperglobulinemia (increases in β- and γ-globulins), resulting in a decreased A/G ratio.

C. Hemostasis (Chapter 4)
1. The vitamin-K-dependent clotting factors (II, VII, IX, and X) and fibrinogen are produced by the liver.
2. Decreased activity of these factors may occur by loss of functional hepatic mass or by interference with their synthetic pathway (vitamin K antagonists or deficiency).
3. The vitamin-K-dependent factors have a relatively short plasma half-life (especially factor VII). Thus, factor deficiencies may be apparent in acute hepatic disease.
4. If clotting factor activity is reduced to less than 30% of baseline activity, clotting tests (prothrombin time, activated partial thromboplastin time) will be prolonged.

BIOCHEMICAL PROFILES OF HEPATIC DISEASES

Different categories of liver diseases tend to display characteristic patterns of biochemical test abnormalities. Although it is often impossible to provide a definitive diagnosis of a specific liver disease based only on serum chemistry profile abnormalities, it may be possible to categorize the general type of liver disease or to exclude some causes of disease.

I. MULTIFOCAL HEPATIC NECROSIS

A. Clinical features and characteristics
1. Microscopic foci of necrosis are dispersed throughout the liver.
2. This pattern of hepatic necrosis usually is associated with infectious diseases.

B. Typical laboratory findings
1. Hepatocellular leakage enzymes have increased serum activity.
2. Analytes affected by alterations in hepatic function or cholestasis may remain within reference intervals or may be increased.

II. CENTRILOBULAR HEPATIC LESIONS CAUSED BY HYPOXIA

A. Clinical features and characteristics
1. Hypoxia primarily affects the centrilobular hepatocytes that are located farthest from the hepatic arterial blood supply and oxygenated blood.
2. Evidence of cardiovascular disease or anemia is present, but clinical signs of hepatic disease usually are absent.
3. High-protein ascites may occur, especially in dogs.

B. Typical laboratory findings
1. The serum activities of hepatocellular leakage enzymes are mildly to moderately increased.

Free ebooks ==> www.Ebook777.com

2. Test results that are affected by cholestasis usually are within the reference interval because bile canaliculi are located at the periphery of the hepatic lobule and are unaffected by the centrilobular lesion.
3. Tests evaluating hepatic function and portal blood flow are usually within reference intervals.
4. Hyperbilirubinemia may be present if tissue hypoxia is the result of hemolytic anemia.

III. ACUTE SUBMASSIVE OR MASSIVE HEPATIC NECROSIS

A. Clinical features and characteristics
1. Sublethal injury or necrosis damages large numbers of hepatocytes.
2. This pattern of disease is typical of a toxic insult to the liver.

B. Typical laboratory findings
1. The serum activity of hepatocellular leakage enzymes is markedly increased due to the large number of hepatocytes affected.
2. Concurrent cholestasis and increases in indicators of cholestatic disease may or may not be present.
3. Hepatic function is often impaired. Albumin concentration usually remains within the reference interval (due to its long half-life), but abnormalities in hemostasis may occur.
4. Tests evaluating portal blood flow are within reference intervals.

IV. LARGE FOCAL HEPATIC LESIONS

A. Clinical features and characteristics
1. These lesions include abscesses, infarcts, or neoplasms that involve only a small portion of the total hepatic mass.
2. Clinical signs of liver disease usually are absent.

B. Typical laboratory findings
1. In acute stages of disease, the serum activity of hepatocellular leakage enzymes may be increased. Mild to moderate increases in enzyme activity are typical because a small portion of the total hepatic mass is involved.
2. With chronic hepatic lesions, serum enzyme activity may be within the reference interval.
3. Other laboratory tests usually are within the reference interval.
4. Hepatic abscesses in ruminants may have polyclonal gammopathy as the only laboratory abnormality.

V. HEPATIC LIPIDOSIS

A. Clinical features and characteristics:
1. This syndrome may occur with diabetes mellitus, acute pancreatitis (cats), hepatotoxicity, inflammatory bowel disease, hyperthyroidism, starvation, or anorexia (particularly in obese cats).
2. Triglycerides accumulate in hepatocytes more rapidly than they can be secreted as lipoproteins.
3. Triglyceride accumulation in hepatocytes leads to hepatocellular swelling and membrane damage. Lipid vacuolation of hepatocytes is visible microscopically in liver biopsies or cytologic fine-needle aspirates.
4. Grossly, the liver is diffusely enlarged and pale.

B. Typical laboratory findings
1. The serum activity of liver leakage enzymes is increased due to altered hepatocellular membrane permeability.

www.Ebook777.com

2. Concentrations of bilirubin and bile acids as well as the activities of ALP and (sometimes) GGT are increased, as intrahepatic cholestasis develops with hepatocellular swelling, causing compression of bile canaliculi.
3. In cats with hepatic lipidosis, ALP activity typically increases to a greater magnitude than does GGT activity, which may remain within reference intervals.
4. Hyperammonemia may occur.
5. Albumin concentration is usually unaffected.
6. Cats with hepatic lipidosis may have prolonged coagulation tests (PT and PTT), but a propensity toward hemorrhage has not been observed clinically.

VI. CORTICOSTEROID HEPATOPATHY

A. Clinical features and characteristics
1. This syndrome occurs in dogs only, and is due to either glucocorticoid treatment or hyperadrenocorcism.
2. Diffuse, indistinct vacuolar change and swelling of hepatocytes results from glycogen accumulation.

B. Typical laboratory findings
1. The serum activity of hepatocellular leakage enzymes is increased.
2. ALP activity is usually increased dramatically.
   a. The corticosteroid ALP isoenzyme is usually involved. This isoenzyme resists levamisole inhibition and heat inactivation.
   b. The hepatic ALP isoenzyme may predominate with recent corticosteroid treatment.
3. Cholestasis may occur from hepatocellular swelling and compression of bile canaliculi.
4. Albumin concentration, ammonia concentration, bile acid concentration, and hemostasis are typically unaffected.

VII. CHOLANGITIS, CHOLANGIOHEPATITIS, AND EXTRAHEPATIC BILIARY DISEASE

A. Clinical features and characteristics
1. Lesions may consist of extrahepatic obstructions with little hepatic involvement or intrahepatic disease affecting the biliary tract.
2. With complete biliary obstruction, acholic feces (pale feces without bile pigments) may be observed.
3. Intermittent or episodic clinical signs may include anorexia, lethargy, pyrexia, vomiting, weight loss, hepatomegaly, and icterus.

B. Typical laboratory findings
1. Bile acid concentration, bilirubin concentration, ALP activity, and GGT activity (tests affected by cholestasis) are increased.
2. Serum activity of hepatocellular leakage enzymes is mildly to moderately increased.
3. Because bile acids are needed for efficient solubilization of dietary lipids, complete biliary obstruction results in malassimilation of fat-soluble vitamin K, which may lead to hemostatic abnormalities.
4. Plasma ammonia concentration is within the reference interval.

VIII. CHRONIC, PROGRESSIVE LIVER DISEASE

A. Clinical features and characteristics

Free ebooks ==> www.Ebook777.com

1. Hepatic lesions may develop from chronic inflammation, toxicosis, or accumulation of metals (e.g., copper, iron), producing progressive, subtle damage to the liver parenchyma.
2. In end-stage liver disease (cirrhosis), normal hepatic architecture is replaced by fibrosis, nodular regeneration of hepatocytes, and bile duct hyperplasia, resulting in disruption of normal portal circulation and biliary drainage.

B. Typical laboratory findings
1. The serum activity of hepatocellular leakage enzymes (ALT, AST, SDH) typically is increased. However, serum enzymatic activity may steadily decline as the disease progresses and the number of affected hepatocytes decreases.
2. Indicators of cholestasis (bilirubin, ALP, GGT, bile acids) usually are increased in concentration or activity.
3. Acquired portosystemic venous shunts may develop due to restriction of portal blood flow and subsequent development of collateral circulatory pathways. As a result, increased concentrations of bile acids and ammonia may be present.
4. Hypoalbuminemia, fasting hypoglycemia, and abnormal hemostasis may be observed if 70% or greater loss of hepatic functional mass occurs.
5. Polyclonal gammopathy is observed commonly because fewer Kupffer cells are present, resulting in decreased clearance of antibodies and/or removal of antigens from portal blood.

IX. CONGENITAL PORTOSYSTEMIC SHUNTS

A. Clinical features and characteristics
1. Portosystemic vascular shunts may be extrahepatic or intrahepatic.
2. Reduced delivery of intestinal and pancreatic hepatotrophic factors to the liver causes hepatic atrophy.
3. Affected animals may display signs of hepatic insufficiency such as weakness from hypoglycemia, edema from severe hypoalbuminemia, and various neurologic abnormalities from hyperammonemia and accumulation of other neurotoxins (hepatic encephalopathy).
4. Hepatic microvascular dysplasia (microscopic portosystemic shunting) may occur as a separate disease or in combination with macroscopic shunts.
5. Dogs with hepatic microvascular dysplasia tend to have less severe disease than those with macroscopic shunts.

B. Typical laboratory findings
1. Baseline plasma ammonia and serum bile acid concentrations usually are increased.
   a. In some animals, baseline bile acid concentrations are within the reference interval.
   b. Postprandial bile acid measurements may be required to demonstrate an abnormality in hepatic function.
2. Markers of cholestasis and hepatocellular leakage usually are within reference intervals.
3. Depending on the degree of hepatic atrophy, reduced hepatic synthesis may result in hypoalbuminemia or decreased BUN concentration.
4. Animals with portosystemic shunts often have microcytic anemia.
5. Animals with acquired portosystemic shunts may have a similar presentation, or may also have increased hepatic enzymes associated with the primary disease causing the shunt.

X. PRIMARY OR METASTATIC HEPATIC NEOPLASIA

A. Clinical features and laboratory findings are variable.

www.Ebook777.com

TABLE 7.1.
TYPICAL CHANGES IN BIOCHEMICAL PROFILES OF ANIMALS WITH VARIOUS HEPATIC DISORDERS.

Disorder	Cytosolic enzymes (ALT, AST, SDH)	Induced enzymes (ALP, GGT)	Bilirubin	Bile acids	Albumin
Multifocal hepatic necrosis	↑[a]	N	N	N	N
Hypoxia/cardiac disease	↑	N	N[b]	N	N
Acute massive necrosis	↑	N to ↑	N to ↑	N to ↑	N
Large focal lesions	N to ↑	N to ↑	N	N	N
Feline hepatic lipidosis	↑	↑[c]	↑	↑	N
Corticosteroid hepatopathy	↑	↑	↑	↑	N
Cholangitis/biliary obstruction	N to ↑	↑	↑	↑	N to ↓
Chronic, progressive liver disease	N to ↑	↑	↑	↑	N to ↓
Congenital portosystemic shunt	N	N	N to ↑	↑	N to ↓
Neoplasia	N to ↑	N to ↑	N to ↑	N to ↑	N to ↓

ALP = alkaline phosphatase
ALT = alanine aminotransferase
AST = aspartate aminotransferase
GGT = gamma glutamyl transferase
SDH = sorbitol dehydrogenase.
[a]N = normal (within the reference interval), ↑ = increased, ↓ = decreased.
[b]If hemolytic disease is the cause of the tissue hypoxia, hyperbilirubinemia will be present.
[c]ALP usually is increased to a greater magnitude than GGT in this condition.

B. Laboratory tests of cholestasis and hepatocellular leakage are typically altered, but the magnitude of the increases do not necessarily correlate with the size or degree of invasiveness of the neoplasm.

C. Laboratory findings in some neoplastic conditions may resemble those of chronic, progressive liver disease or large, focal, hepatic lesions (see above) (Table 7.1).

REFERENCES

Anwer MS, Meyer DJ: 1995. Bile acids in the diagnosis, pathology, and therapy of hepatobiliary diseases. *Vet Clin North Am Small Anim Pract* 25:503–517.

Bayly WM, Brobst DF, Elfers RS, et al: 1986. Serum and urinary biochemistry and enzyme changes in ponies with acute renal failure. *Cornell Vet* 6:306–316.

Britti D, Massimini G, Peli A, et al: 2005. Evaluation of serum enzyme activities as predictors of passive transfer status in lambs. *J Am Vet Med Assoc* 226:951–955.

Bunch SE: 1993. Hepatotoxicity associated with pharmacologic agents in dogs and cats. *Vet Clin North Am Small Animal Pract* 23:659–670.

Center SA, Baldwin BH, Dillingham S, et al: 1986. Diagnostic value of serum gamma-glutamyl transferase and alkaline phosphatase activities in hepatobiliary disease in the cat. *J Am Vet Med Assoc* 188:507–510.

Free ebooks ==> www.Ebook777.com

Center SA, Crawford MA, Guida L, et al: 1993. A retrospective study of 77 cats with severe hepatic lipidosis: 1975–1990. *J Vet Intern Med* 7:349–359.

Center SA: 1995. Pathophysiology and Laboratory Diagnosis of Hepatobiliary Disorders. *In:* Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*, 4th ed. WB Saunders, Philadelphia, pp. 1261–1312.

Center SA: 1999. Chronic liver disease: Current concepts of disease mechanisms. *J Small Animal Pract* 40:106–114.

Christiansen JS, Hottinger HA, Allen L, et al: 2000. Hepatic microvascular dysplasia in dogs: a retrospective study of 24 cases (1987–1995). *J Am Anim Hosp Assoc* 36:385–389.

Craig AM, Pearson EG, Rowe K: 1992.Serum bile acid concentrations in clinically normal cattle: Comparison by type, age, and stage of lactation. *Am J Vet Res* 53:1784–1786.

Dial SM: 1995. Clinicopathologic evaluation of the liver. *Vet Clin North Am Small Anim Pract* 25:257–273.

Garzotto CK, Berg J, Hoffmann WE, et al: 2000. Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. *J Vet Intern Med* 14:587–592.

Gieger TL, Hosgood G, Taboada J, et al: 2000. Thyroid function and serum hepatic enzyme activity in dogs after phenobarbital administration. *J Vet Intern Med* 14:277–281.

Guglick MA, MacAllister CG, Ely RW, et al: 1995. Hepatic disease associated with administration of tetanus antitoxin in eight horses. *J Am Vet Med Assoc* 206:1737–1740.

Harr KE: 2002. Clinical chemistry of companion avian species: a review. *Vet Clin Pathol* 31:140–151.

Hoffmann WE, Baker G, Rieser S, Dorner JL: 1987. Alterations in selected serum biochemical constituents in equids after induced hepatic disease. *Am J Vet Res* 48:1343–1347.

Hoffman WE, Solter PF: 2008. Diagnostic Enzymology in Domestic Animals. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds.): *Clinical Biochemistry of Domestic Animals*, 6th ed. Academic Press, San Diego, pp. 351–378.

Horney BS, Honor DJ, MacKenzie A, et al: 1993. Stability of sorbitol dehydrogenase activity in bovine and equine sera. *Vet Clin Pathol* 22:5–9.

Horney BS, Farmer AJ, Honor DJ, et al: 1994. Agarose gel electrophoresis of alkaline phosphatase isoenzymes in the serum of hyperthyroid cats. *Vet Clin Pathol* 23:98–102.

Lawler DF, Keltner DG, Hoffman WE, et al: 1996. Benign familial hyperphosphatasemia in Siberian huskies. *Am J Vet Res* 57:612–617.

Lumeij JT, Westerhof I: 1987. Blood chemistry for the diagnosis of hepatobiliary disease in birds. A review. *Vet Quarterly* 9:255–261.

Lumeij, JT: 1994. Hepatology. In: Ritchie BW, Harrison GJ, Harrison LR (eds): *Avian Medicine: Principles and Application*. Wingers Publishing, Inc., Lake Worth, FL, pp. 523–537.

Mahaffey EA, Lago MP: 1991. Comparison of techniques for quantifying alkaline phosphatase isoenzymes in canine serum. *Vet Clin Pathol* 20:51–55.

Martin RA: 1993. Congenital portosystemic shunts in the dog and cat. *Vet Clin North Am Small Anim Pract* 23:609–623.

McGorum BC, Murphy D, Love S, et al: 1999. Clinicopathological features of equine primary hepatic disease: A review of 50 cases. *Vet Rec* 145:134–139.

www.Ebook777.com

Muller PB, Taboada J, Hosgood G, et al: 2000. Effects of long-term phenobarbital treatment on the liver in dogs. *J Vet Intern Med* 14:165–171.

Nestor DD, Holan KM, Johnson CA, et al: 2006. Serum alkaline phosphatase activity in Scottish Terriers versus dogs of other breeds. *J Am Vet Med Assoc* 228:222–224.

Rothuizen J, van den Ingh T: 1988. Covalently protein-bound bilirubin conjugates in cholestatic disease of dogs. *Am J Vet Res* 49:702–704.

Rutgers HC, Batt RM, Vaillant C, et al: 1995. Subcellular pathologic features of glucocorticoid-induced hepatopathy in dogs. *Am J Vet Res* 56:898–907.

Sanecki RK, Hoffmann WE, Gelberg HB, et al: 1987. Subcellular location of corticosteroid-induced alkaline phosphatase in canine hepatocytes. *Vet Pathol* 24:296–301.

Saulez MN, Cebra CK, Tornquist SJ: 2004. The diagnostic and prognostic value of alkaline phosphatase activity in serum and peritoneal fluid from horses with acute colic. *J Vet Intern Med* 18:564–567.

Solter PF, Hoffmann WE: 1999. Solubilization of liver alkaline phosphatase isoenzyme during cholestasis in dogs. *Am J Vet Res* 60:1010–1015.

Solter PF, Hoffmann WE, Hungerford LL, et al: 1993. Assessment of corticosteroid-induced alkaline phosphatase isoenzyme as a screening test for hyperadrenocorticism in dogs. *J Am Vet Med Assoc* 203:534–538.

Sterczer A, Gaal T, Perge E, Rothuizen J: 2001. Chronic hepatitis in the dog—A review. *Vet Quarterly* 23:148–152.

Swenson CL, Graves TK: 1997. Absence of liver specificity for canine alanine aminotransferase (ALT). *Vet Clin Pathol* 26:26–8.

Tennant BC, Center SA: 2008. Hepatic Function. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds.): *Clinical Biochemistry of Domestic Animals*, 6th ed. Academic Press, San Diego, pp. 379–412.

Webster CRL: 2004. History, clinical signs, and physical findings in hepatobiliary disease. *In:* Ettinger SJ (ed): *Textbook of Veterinary Internal Medicine*. 6th ed. W.B. Saunders Co., Philadelphia, pp. 1422–1435.

West HJ: 1996. Clinical and pathological studies in horses with hepatic disease. *Equine Vet J* 28:146–156.

West HJ: 1997. Clinical and pathological studies in cattle with hepatic disease: *Vet Res Commun* 21:169–185.

Free ebooks ==> www.Ebook777.com

CHAPTER 8

DIGESTIVE SYSTEM

Heather L. Tarpley, DVM, and Denise I. Bounous, DVM, PhD

EXOCRINE PANCREAS

Laboratory evaluation of the exocrine pancreas is directed toward two categories of disease: inflammation and necrosis, and exocrine insufficiency.

LABORATORY DETECTION OF PANCREATITIS

Pancreatitis entails the destruction of pancreatic tissue by activated digestive enzymes. The clinical spectrum of pancreatitis includes mild subclinical disease, severe necrotizing inflammation that can be life threatening, and clinical disease that is episodic over months to years. Acute and chronic pancreatitis are histopathological categories which typically cannot be distinguished by physical examination findings or laboratory test results. Both acute and chronic disease can be mild or severe. In domestic species, pancreatitis occurs primarily in the dog and cat, though it also has been reported in the horse, cow, and bird. A presumptive diagnosis of pancreatitis requires the integration of clinical presentation, laboratory abnormalities, and imaging (ultrasonography and/or radiology). A definitive diagnosis requires histological examination of pancreatic tissue. Histologic evidence of suppurative inflammation, necrosis, and edema are diagnostic for acute pancreatitis. Evidence of permanent pancreatic structural damage, especially fibrosis, and mononuclear (lymphocytic) inflammation are characteristics of chronic disease. "Chronic active pancreatitis" can be used to describe the presence of histologic changes characteristic of both acute and chronic pancreatitis.

Routine biochemical profiles lack highly sensitive and specific tests for pancreatic disease and therefore cannot be used for definitive diagnosis of pancreatitis. Nevertheless, a complete blood count, biochemical profile (including lipase and amylase measurement in dogs), and urinalysis in addition to specific tests for pancreatic disease are recommended for patients for whom pancreatitis is a differential diagnosis. Pancreatitis is frequently associated with disease of other body systems. General laboratory testing facilitates awareness of dysfunction or illness of other organs.

I. SERUM ENZYME ACTIVITY MEASUREMENT IN GENERAL BIOCHEMICAL PROFILES

A. Enzymes or zymogens (inactive precursors of enzymes) produced and stored in acinar cells may leak into plasma during pancreatic cellular injury, resulting in increased serum activity. Later in the course of disease, decreased serum enzyme activity may occur from depletion of stored enzymes or disturbed enzyme synthesis. Amylase and lipase are enzymes that are nonexclusively produced by the

www.Ebook777.com

exocrine pancreas. Measurement of their activity in serum can be helpful in determining the presence of pancreatitis in dogs; their measurement is of no utility in cats.

B. Serum amylase activity

1. Characteristics

a. Only α-amylase is present in animals. It is secreted in the active form, and it hydrolyzes complex carbohydrates at α-1,4-linkages to form maltose and glucose.

b. Serum amylase activity is expressed in units per liter (U/L).

c. Pancreas, liver, and small intestine are the main sources of serum amylase activity.

d. Of these sources, the duodenal mucosa is the most likely origin of serum amylase activity in health. Because pancreatectomy does not result in decreased serum amylase activity, most enzymatic activity must originate from extrapancreatic sources.

e. Salivary amylase is not present in domestic animals except the pig.

f. Amylase is filtered by the glomerulus and resorbed and inactivated by renal tubular epithelium; very little amylase activity is detected in the urine.

g. Kupffer cells in the liver also resorb and inactivate small quantities of amylase.

h. In dogs, serum amylase activity may remain within the reference interval in some cases of acute pancreatitis. More false-negative test results occur with serum amylase than with serum lipase.

i. Studies have shown that corticosteroid administration may decrease serum amylase activity.

j. Lipemia in the patient's serum sample may inhibit amylase activity. This may be overcome by sample dilution or serum clearing techniques.

2. Interpretation of hyperamylasemia (Table 8.1)

a. Hyperamylasemia is often present in dogs with pancreatitis; it is rarely observed in cats. Birds with pancreatitis also may have hyperamylasemia.

TABLE 8.1
CAUSES OF HYPERAMYLASEMIA AND HYPERLIPASEMIA

Hyperamylasemia
Pancreatitis
Renal disease
Diseases resulting in production/decreased clearance of macroamylase
Diabetes mellitus
Gastrointestinal disease
Neoplasia
Hepatobiliary disease
Surgical manipulation of the pancreas
Hyperlipasemia
Pancreatitis
Renal disease
Peritonitis
Gastritis/enteritis
Hepatic disease
Bowel obstruction
Visceral manipulation during surgery
Neoplasia (may be profound)
Corticosteroids

Free ebooks ==> www.Ebook777.com

(1) Degenerating pancreatic acinar cells or obstructed pancreatic ducts cause leakage of amylase directly into venules or indirectly into the blood via lymphatics.

(2) The higher the amylase activity (equal to or greater than three- to four-fold increase), the more likely the presence of pancreatic disease. Values 7 to 10 times the reference interval have been observed with pancreatitis.

(3) Amylase activity peaks at 12 to 48 hours and returns to the reference interval within 8 to 14 days after a single episode of pancreatitis in dogs.

(4) Ranges of sensitivity and specificity of serum amylase activity for pancreatitis in dogs are reportedly 62% to 78% and 57% to 77%, respectively.

b. Hyperamylasemia has poor specificity for pancreatitis. Nonpancreatic diseases that also can be associated with hyperamylasemia include renal disease, diabetes mellitus, gastrointestinal disease, neoplasia (e.g., lymphosarcoma and hemangiosarcoma), and hepatobiliary disease.

(1) Serum amylase measurement includes macroamylase, a complex of amylase bound to immunoglobulin.

(a) Renal filtration of these complexes is hindered by their size.

(b) Macroamylase, which can account for 5% to 62% of total amylase activity, is found in higher concentration in the serum of dogs with renal disease and/or a decreased glomerular filtration rate and consequently contributes to the hyperamylasemia seen in renal disease.

(2) Nonpancreatic diseases that affect serum amylase concentration typically result in mild to moderately increased activity.

(3) Because diseases other than pancreatitis may cause hyperamylasemia, both serum amylase and lipase activities should be measured in dogs to better substantiate a suspicion of pancreatitis. Increases and decreases in serum amylase activity tend to parallel those of lipase in pancreatic disease.

C. Lipase

1. Characteristics

a. Lipase hydrolyzes triglycerides.

b. Serum lipase activity is expressed in units per liter (U/L).

c. Lipase activity in serum originates from multiple sources including the pancreas and stomach. The common function of lipases precludes their differentiation by assays determining serum lipase activity.

(1) Serum lipase activity in pancreatectomized dogs has been shown to be 50% of its level prior to surgery.

(2) Serum lipase activity in dogs with exocrine pancreatic insufficiency and healthy dogs has been shown to not be significantly different.

d. Lipase is cleared from the plasma and inactivated by the kidneys.

e. Hemolysis directly inhibits lipase activity.

2. Interpretation of hyperlipasemia (Table 8.1)

a. In dogs, a three-fold or greater increase in serum lipase activity suggests acute pancreatitis.

(1) In dogs with pancreatitis, lipase activity increases within 24 hours and peaks (at a higher level of activity than amylase) two to five days after onset.

(2) Sensitivity and specificity of serum lipase activity for canine pancreatitis are not high; ranges are 60% to 75% and 50% to 75%, respectively.

b. Decreased clearance of plasma lipase in renal disease can result in a two- to three-fold increase in serum lipase activity.

c. Peritonitis, gastritis, enteritis, some hepatic disorders, bowel obstruction, visceral manipulation during laparotomy, and neoplasia also may increase serum lipase activity.

(1) Lipase activity may be elevated two- to three-fold above the reference interval.

(2) The source of increased lipase activity is unknown.

www.Ebook777.com

d. Corticosteroid administration may increase serum lipase activity up to five times the upper end of the reference interval in the absence of histologic evidence of pancreatitis. The source of increased lipase activity associated with corticosteroid use has not been determined.

e. Specific tumors have been associated with hyperlipasemia.

(1) Marked to profound hyperlipasemia (11 to 93× the upper reference limit) may be observed in dogs with pancreatic and hepatic carcinomas.

(2) Bile duct carcinoma and gastrointestinal lymphosarcoma have also been associated with increased serum lipase activity.

II. LABORATORY TESTS SPECIFIC FOR PANCREATIC DISEASE

A. Species-specific pancreatic lipase immunoreactivity (PLI)

1. PLI is a measurement of serum lipase derived only from the exocrine pancreas; it is measured in µg/L.

2. PLI has been shown to be the most sensitive and specific laboratory test currently available to veterinary practitioners for diagnosis of pancreatitis in both dogs and cats.

3. Assays have been developed and validated for both the dog and cat.

a. Commercial assays for species-specific pancreatic lipase based on PLI technology (Spec cPL™ and Spec fPL™) are currently available through the Gastrointestinal Laboratory at Texas A & M University, College of Veterinary Medicine, College Station, TX 77843 (gilab@cvm.tamu.edu) and IDEXX Reference Laboratories.

(1) Sensitivity and specificity of canine PLI for pancreatitis are reportedly 81% and 96%, respectively.

(2) Sensitivity and specificity of feline PLI for pancreatitis are reportedly 67% and 91%, respectively (when measured by radioimmunoassay). These parameters of fPLI increase with severity of disease.

(a) fPLI has a sensitivity of 54% in cats with mild or low-grade/chronic pancreatitis. This level of sensitivity is higher than any other noninvasive test for pancreatitis (including serum amylase and lipase activity, feline trypsin-like immunoreactivity, radiography, abdominal ultrasound, and computed tomography).

(b) One study found sensitivity and specificity of fPLI to be 100% in cases of moderate to severe pancreatitis.

b. A "pet-side" test for canine pancreatic lipase, the IDEXX SNAP® cPL™, is also available and has 95% correlation with PLI measured through reference laboratories.

(1) Test results are considered either "normal" or "abnormal."

(2) Measurement of PLI through a reference laboratory is recommended for dogs with abnormal test results for the sake of comparison during the course of treatment.

(3) A similar test kit is currently not available for cats.

4. Glomerular filtration rate, gastritis, and oral prednisone have been shown to not affect cPLI results.

5. In cats with experimentally induced pancreatitis, fPLI was shown to remain elevated longer than feline trypsin-like immunoreactivity (fTLI).

6. fPLI measurement could be of great importance for cats with other diseases.

a. Pancreatitis in cats is frequently associated with other conditions (e.g., hepatic lipidosis, diabetes mellitus, cholangitis, cholangiohepatitis, inflammatory bowel disease, interstitial nephritis, and vitamin-K-responsive coagulopathy).

b. Routine biochemical profiles may reveal abnormalities related to disease of other organs but do not disclose evidence of pancreatitis. Identification of pancreatitis in cats with disease elsewhere is potentially clinically relevant.

c. A recent study has shown that a significant number of cats with diabetes mellitus have elevated fPLI values. Measurement of fPLI in diabetic cats is recommended, especially when the diabetic condition has been difficult to control.

Free ebooks ==> www.Ebook777.com

B. Trypsin-like immunoreactivity (TLI)

1. TLI is a species-specific assay that detects trypsinogen and trypsin in serum.

2. Trypsinogen and trypsin have been shown to originate almost exclusively from the exocrine pancreas in dogs. Though studies have not been performed, the origin of these proteins in cats is believed to be only the pancreas. TLI, therefore, is highly specific for pancreatic disease. TLI measurement is an indirect test of pancreatic function.

3. In health, only a small amount of trypsinogen and very little trypsin are secreted into the vascular space. Pancreatic injury/inflammation results in increased serum levels of trypsinogen and trypsin.

4. Sensitivity and specificity of canine TLI are approximately 35% and 65%, respectively.

5. Overall sensitivity and specificity of feline TLI for pancreatitis are approximately 30% and 75%, respectively.

6. Low sensitivity of TLI excludes its use as a definitive test for pancreatitis for both dogs and cats.

a. The short half-life of TLI likely decreases its sensitivity. TLI may return to the reference interval within several hours of onset of pancreatitis. Utility of TLI is highest when measured as soon as clinical signs of disease are recognized.

b. In chronic pancreatitis, the concentration of enzymes in serum may be inadequate for detection.

7. TLI may be increased with renal insufficiency.

8. Increased TLI also has been reported in malnourished dogs without histologic evidence of pancreatitis.

9. In summary, serum TLI can be useful in the diagnosis of pancreatitis if renal disease is not present. The magnitude of increased TLI does not always correlate with the severity of pancreatitis.

10. TLI measurement is currently only available at reference laboratories.

C. Trypsinogen activation peptide (TAP)

1. TAP is produced after cleavage of trypsinogen by enterokinase within the small intestine. This peptide is predominantly found within the intestinal lumen, but it also is increased in plasma and the abdominal cavity if trypsinogen is aberrantly activated within an inflamed pancreas. The kidneys ultimately clear TAP from circulation.

2. Marked elevations in serum TAP occur with severe pancreatitis and severe renal disease. If renal disease is excluded, markedly increased TAP could be considered a negative prognostic indicator; intensive therapy would be warranted.

3. TAP fails to increase in mild pancreatitis, limiting its usefulness as the sole marker of pancreatitis in dogs.

4. Limited availability of testing and inadequate scientific evaluation of the use of serum and urine TAP as tests for pancreatitis make their measurement as parts of a diagnostic strategy for pancreatitis in dogs and cats impracticable.

III. SUPPLEMENTARY LABORATORY FINDINGS IN PANCREATIC INFLAMMATION AND NECROSIS (CASE 14)

A. The complete blood count

1. Mild polycythemia or a mild to moderate anemia can be present. Internal fluid shifts and fluid loss via vomiting may cause a relative polycythemia. Anemia observed with pancreatitis is usually nonregenerative as a result of inflammation or chronicity of disease.

2. The leukogram can vary widely.

a. Mild leukocytosis attributable to mild neutrophilia, marked leukocytosis with a left shift, and leukopenia with a degenerative left shift have all been observed with pancreatitis.

b. Leukocytosis is most commonly seen with acute necrotizing pancreatitis or pancreatic abscessation.

www.Ebook777.com

c. Neutrophils may exhibit toxic changes (Chapter 2).

d. Lymphopenia, eosinopenia, and monocytosis can be aspects of a corticosteroid or "stress" leukogram which results from increased endogenous cortisol production induced by disease.

3. Thrombocytopenia observed with pancreatitis may be attributable to disseminated intravascular coagulation triggered by release of digestive enzymes into circulation.

B. Serum biochemical abnormalities

1. Fasting hyperlipidemia

a. Hypertriglyceridemia (hyperlipemia)

(1) Lipoprotein lipase, a plasma lipemia-clearing enzyme produced by the pancreas, may be inactivated in pancreatic necrosis, resulting in a transient lipemia.

(2) Diabetes mellitus as a sequela to pancreatitis may cause hyperlipemia.

b. Hypercholesterolemia

2. Hyperproteinemia (usually relative) or hypoproteinemia (including hypoalbuminemia) may be observed.

3. Prerenal and renal azotemia may result from a decreased glomerular filtration rate due to dehydration.

4. Increased ALT and AST activities may occur from hepatic ischemia or the toxic effects of products absorbed by portal blood.

5. Increased ALP activity and hyperbilirubinemia may result from cholestasis secondary to hepatocellular fatty change or inflammation causing compression of bile canaliculi or the common bile duct.

6. Hyperglycemia may occur secondary to the following:

a. Alpha cells in the pancreatic islets of Langerhans release glucagon. Hyperglucagonemia subsequently may promote mobilization of glycogen stores.

b. Catecholamine and cortisol release from the adrenal medulla and cortex, respectively, may promote glycogenolysis and gluconeogenesis resulting in transient hyperglycemia.

c. Transient or persistent diabetes mellitus may occur from damage to insulin-producing beta cells within pancreatic islets.

7. Increased C-reactive protein

a. C-reactive protein (CRP), a positive acute phase protein produced in response to inflammation, infection, and tissue destruction (including necrosis caused by trauma), is commonly increased in cases of acute pancreatitis.

b. In inflammatory conditions, CRP may be elevated before leukocytosis and/or a left shift is detected.

c. The utility of CRP as a predictor of severity of pancreatitis or as an indicator of response to treatment has not been critically evaluated.

8. Electrolytes vary in concentration, though they are commonly below the reference interval.

a. Hypocalcemia and hypokalemia are the most common electrolyte abnormalities.

b. The mechanism of hypocalcemia is unclear, but saponification of fat or loss of albumin, and consequently albumin-bound calcium, into an effusion may be responsible for this abnormality.

c. Prognosis worsens with hypocalcemia, especially when the ionized calcium is less than 1.0 mmol/L. Ionized hypocalcemia associated with pancreatitis is usually observed with severe disease.

C. Urinalysis

1. Glomerular damage via excessive filtration of pancreatic enzymes may result in proteinuria.

2. Persistent glycosuria may indicate concurrent diabetes mellitus.

D. Peritoneal fluid evaluation

1. A serosanguineous peritoneal effusion may be present and is characterized by lipid droplets, erythrocytes, and neutrophils (nonseptic exudate).

Free ebooks ==> www.Ebook777.com

2. Increased amylase and lipase activities in peritoneal effusion fluid may exceed activities of these enzymes in serum.

E. Studies have consistently shown that pancreatitis in cats, especially chronic disease, is frequently observed with other pathologies. The most common coexistent diseases in cats with pancreatitis include hepatobiliary disease, renal disease, inflammatory bowel disease, and diabetes mellitus.

IV. DIAGNOSTIC STEPS FOR CANINE AND FELINE PANCREATITIS

A. Dogs

1. Have suspicion in a dog presented for vomiting, cranial abdominal pain, or fever that is a predisposed breed (e.g., Miniature Schnauzer) or that has a recent history of a diet change, dietary indiscretion, or ingestion of a fatty meal.
2. Perform standard laboratory testing (complete blood count, biochemical profile including amylase and lipase, and urinalysis).
3. Measure canine PLI via serum submission to a reference laboratory or perform an in-hospital SNAP® cPL™ test. PLI offers the highest sensitivity and specificity of any noninvasive test for pancreatitis.
4. Consider TLI measurement. TLI has reasonable specificity but lacks adequate sensitivity to be a primary test for pancreatitis.
5. Ultrasonographic evaluation may aid diagnosis.
6. Comparison of serum and peritoneal amylase activities may provide supportive evidence of pancreatitis. In cases of pancreatitis, peritoneal amylase activity is greater than that of serum.
7. Though histologic evaluation of pancreatic tissue can provide a definitive diagnosis, there is increased anesthetic risk in animals with pancreatitis.

B. Cats

1. Have heightened concern for pancreatitis in cats presented for nonspecific findings of lethargy, anorexia, dehydration, and weight loss for which there is no known cause.
2. Test for feline leukemia virus and feline immunodeficiency virus if the cat is unvaccinated or its status is unknown.
3. Perform standard laboratory testing (complete blood count, biochemical profile, and urinalysis). Though it is possible in cats with pancreatitis to have all results of these standard tests to be within reference intervals, performance of the tests aids elucidation of the presence of other diseases and gives an impression of the patient's general condition.
4. Measure feline PLI by a reference laboratory. fPLI is the most sensitive and specific noninvasive test available for diagnosing feline pancreatitis.
5. Consider testing for hyperthyroidism in cats older than six years.
6. Abdominal radiography and/or ultrasonography may be helpful, though test sensitivities are inadequate for definitive diagnosis.
7. Thoracic radiography should be performed if pleural effusion secondary to pancreatitis is suspected.
8. Hepatic function and gastrointestinal screening tests (e.g., bile acids or urine sulfated bile acids and cobalamin/folate measurements) are helpful in ascertaining the existence of abnormalities in other organs.
9. Cobalamin measurement is recommended for cats in which pancreatitis is suspected given the commonality of coexistent intestinal disease with pancreatitis and improved overall response to therapy when hypocobalaminemia is recognized and managed.
10. Histologic evaluation of pancreatic tissue may provide a definitive diagnosis, but surgery carries the same risk in cats as it does in dogs that have pancreatitis.
11. Some experts also recommend measurement of IgG and IgM titers for *Toxoplasma gondii* in cats that potentially have been exposed.

www.Ebook777.com

12. Measurement of serum amylase and lipase activities and computed tomography are of no utility in the diagnosis of feline pancreatitis.

13. Studies have demonstrated in cats an association of pancreatitis, inflammatory bowel disease, and biliary tract disease ("triaditis" is a designation for the concurrence of all three). Bear in mind that a cat with one of these disorders is at risk for having or developing one or both of the remaining conditions.

LABORATORY DETECTION OF MALASSIMILATION ATTRIBUTABLE TO MALDIGESTION (EXOCRINE PANCREATIC INSUFFICIENCY)

Malassimilation of nutrients from the gastrointestinal lumen is secondary to defective digestion or malabsorption. Maldigestion principally is caused by exocrine pancreatic insufficiency (EPI). Malabsorption typically results from small intestinal disease. EPI occurs when at least 90% of the exocrine pancreas is nonfunctional. Subsequent severe loss of digestive enzyme synthesis results in maldigestion followed by malassimilation of nutrients. Typical clinical signs of EPI are weight loss, steatorrhea, diarrhea, and polyphagia; these signs are indistinguishable from those observed with disorders of malabsorption. A minority of cats with EPI may only exhibit weight loss.

I. EPI IN DOGS AND CATS

A. The pathogenesis of EPI in dogs is unclear, though immune-mediated destruction of pancreatic acini is suspected.

B. EPI in dogs is most commonly recognized in young dogs and is an inherited autosomal recessive condition in the German Shepherd, Rough-coated Collie, and other breeds.

C. EPI also can be seen in older animals secondary to pancreatic neoplasia, chronic pancreatitis, and immune-mediated disease.

D. Rate of development of EPI secondary to pancreatic destruction via inflammation depends on the frequency and severity of episodes of pancreatitis.

E. EPI in cats is caused most frequently by chronic pancreatitis.

II. DIAGNOSIS OF EPI

Diagnosis of EPI in both dogs and cats rests on compatible clinical signs and abnormal pancreatic function test results.

A. Serum trypsin-like immunoreactivity (TLI)

1. To date, measurement of serum TLI concentration is the noninvasive test of choice for both canine and feline EPI.

2. Because serum TLI is dependent upon pancreatic mass, a reduction in its mass, as occurs in EPI via pancreatic atrophy, leads to reduced serum TLI concentration (Case 15).

3. Though TLI is considered a sensitive test, false-negative test results for EPI may occur.

a. Coexisting pancreatitis may increase the amount of TLI released into plasma. TLI may then be within or above the reference interval though EPI is present.

(1) The overall incidence then of EPI is likely much greater than realized in animals that also have chronic pancreatitis.

(2) Other pancreatic function tests, e.g., measurement of fecal proteolytic activity (see below), may be used in cases for which there is suspicion of EPI but TLI results are within the reference interval.

Free ebooks ==> www.Ebook777.com

(3) Diagnoses of coincident EPI and diabetes mellitus increase suspicion of underlying chronic pancreatitis.

b. Renal failure also may result in a false-negative TLI test result.

(1) Filtered trypsinogen is catabolized by tubular epithelial cells.

(2) Reduced renal filtration prolongs the serum circulating half-life of TLI. Consequently, TLI concentration may be within the reference interval even if EPI is present.

4. TLI assays require animals to be fasted for 12 hours. Oral supplementation of pancreatic enzymes does not affect serum TLI concentrations.

5. Though both canine TLI and PLI have been shown to each have high sensitivity in the diagnosis of EPI, canine TLI measurement has greater specificity than canine PLI for EPI. A small degree of overlap observed in PLI measurements in dogs with EPI and healthy dogs substantiates the "gold standard" status of TLI testing for EPI.

6. Persistent TLI measurements in healthy dogs between the cut-off and lower end of the reference interval is consistent with subclinical EPI and partial pancreatic atrophy. These animals typically do not have diarrhea and could be subclinical for years or for life.

B. Fecal proteolytic activity

1. Fecal proteases are primarily of pancreatic origin and include trypsin, chymotrypsin, and carboxypeptidases A and B.

a. Proenzymes are secreted by the pancreas and are activated to functional enzymes in the duodenum.

b. Trypsinogen is activated to trypsin by enterokinase produced by the duodenal mucosa. Trypsin subsequently activates the other proteases.

2. Measurement of fecal proteolytic activity (via azocasein hydrolysis test, radial enzyme diffusion assay, X-ray film digestion, or gelatin-tube test) can be helpful in the diagnosis of EPI but is cumbersome.

a. Fecal samples on at least three consecutive days should be collected and then stored and shipped frozen overnight for evaluation at a reference laboratory.

b. Fluctuations in fecal proteolytic activity can lead to a false-positive diagnosis of EPI.

c. Fecal proteolytic testing at reference laboratories is limited.

3. Availability of reliable species-specific TLI measurement minimizes the utility of fecal proteolytic activity measurement in the diagnosis of EPI in dogs and cats.

4. In species for which a TLI assay has not been developed, measurement of fecal proteolytic activity can be helpful, primarily in excluding EPI as a cause for chronic diarrhea.

5. In cases of protein-losing enteropathy (PLE), α_1 proteinase inhibitor (α1-PI), a serum protein, is lost into the intestinal lumen. A study has demonstrated that increased fecal α1-PI concentration can result in decreased fecal proteolytic activity, and consequently, in false-positive diagnoses of EPI.

C. Measurements of cobalamin (vitamin B_{12}) and folate (see below) are strongly recommended because abnormalities in these parameters are frequently observed in dogs and cats with EPI and lack of their correction may compromise a proper response to treatment for EPI.

D. Histologic changes observed in biopsies of affected pancreata can be suggestive of but not diagnostic for EPI.

E. The bentiromide (N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid [BT-PABA]) absorption test historically was used for detection of EPI. This test largely has been replaced by species-specific TLI.

LABORATORY DETECTION OF MALASSIMILATION ATTRIBUTABLE TO MALABSORPTION

Malabsorption occurs with bacterial overgowth, interference with venous, lymphatic drainage (lymphangiectasia), intestinal lesions characterized by decreased absorptive surface area (e.g., villous

www.Ebook777.com

atrophy or loss), death of epithelial cells, diffuse inflammatory disease (e.g., lymphoplasmacytic, eosinophilic, and granulomatous enteritides), and diffuse intestinal neoplasia (chiefly intestinal lymphoma). Most common clinical signs of small intestinal malabsorptive disease in dogs and cats (diarrhea, weight loss, and steatorrhea) are similar to those associated with maldigestion. Diarrhea may not always be seen in the horse or cat with small intestinal malabsorptive disease due to the lower intestinal tract's absorptive capacities. Diseases associated with vitamin deficiencies attributable to malabsorption, such as secondary hyperparathyroidism and bleeding diathesis via vitamin D and vitamin K deficiencies, respectively, have been documented in veterinary species.

Definitive diagnosis of most causes of malabsorption requires histologic evaluation of affected tissue biopsied via endoscopy or laparotomy. Many tests, however, have been developed and used to screen for defective mucosal absorption. Currently, the most common laboratory tests related to malabsorption are serum cobalamin (vitamin B_{12}) and folate, which are micronutrients absorbed at specific sites in the intestinal tract. Serum concentrations of cobalamin and folate reflect a balance between dietary intake, bacterial utilization and production, absorption, and body losses. Fecal smear analyses for nutrients, various absorption tests (including plasma turbidity test, glucose absorption test, D-xylose absorption test, and fecal fat quantitation) were historically used to detect malabsorption but are beleaguered with inadequate sensitivity and/or specificity, complicated testing protocols, and limited availability of reference laboratory evaluation. These latter tests, the details of which were outlined in the previous edition, are rarely used in the veterinary practice setting. Instances in which absorption tests might be useful include cats with idiopathic weight loss without diarrhea, when trying to determine the significance of mild infiltrates of inflammatory cells observed in canine intestinal biopsies, and in the rare case of disaccharidase deficiency in young animals.

Clinical investigation of diarrhea in general should include fecal parasitology. Testing for giardiasis, cryptosporidiosis, and clostridial enterotoxicosis should be considered. Microbiological culture also can aid diagnosis. A complete blood count, biochemical profile, urinalysis, TLI measurement, and possible liver function testing are recommended in chronic unresponsive diarrheas.

I. SMALL INTESTINAL BACTERIAL OVERGROWTH (SIBO)

A. SIBO is an over-proliferation of bacteria in the duodenum and jejunum most commonly recognized in dogs and humans that can result in malabsorption by multiple and complex mechanisms.

B. SIBO can be primary (idiopathic, also referred to as antibiotic-responsive diarrhea when other disease processes have been excluded) or associated with a number of conditions, including exocrine pancreatic insufficiency, inflammatory bowel disease, intestinal stagnation or deranged motility, partial or complete intestinal obstruction, lymphoma, lymphangiectasia, immunodeficiencies (resulting in impaired mucosal defense mechanisms), decreased gastric acid secretion, gastric surgery, and resection of the ileocecocolic valve.

C. Idiopathic SIBO predominantly affects young animals; German Shepherd dogs are overrepresented.

D. A clear consensus of diagnostic criteria for SIBO (both primary and secondary) in domestic animals currently does not exist.

1. For some veterinary internists and investigators, culture and quantitation of intestinal bacteria are considered definitive tests for diagnosing SIBO.

2. Quantitation of intestinal bacteria is difficult, expensive, and available only at some large institutions.

3. Less invasive screening tests (e.g., fasting serum cobalamin and folate measurements and serum unconjugated cholic acid concentration; see below) or observation for a response to therapy, however, are the primary means used in a practice setting to obtain supportive evidence of SIBO.

E. Serum cobalamin (vitamin B_{12}, Table 8.2)

Free ebooks ==> www.Ebook777.com

TABLE 8.2
TYPICAL LABORATORY CHANGES IN FOLATE CONCENTRATION, COBALAMIN CONCENTRATION, AND TRYPSIN-LIKE IMMUNOREACTIVITY IN VARIOUS MALASSIMILATION DISORDERS.

Disorder	Folate	Cobalamin	Trypsin-like immunoreactivity (TLI)
Exocrine pancreatic insufficiency	↑ or [a]N	↓	↓
Bacterial overgrowth	↑	↓	N
Proximal small intestinal disease	↓	N	N
Distal small intestinal disease	N	↓	N
Diffuse small intestinal disease	↓	↓	N

[a]N = normal (within the reference interval)
↑ = increased
↓ = decreased

1. Normal absorption
   a. Cobalamin is readily available in canine diets; dietary deficiency is unlikely.
   b. Cobalamin is liberated from dietary protein in the stomach by acid and pepsin.
   c. The free vitamin is bound to haptocorrin, a nonspecific cobalamin-binding protein.
   d. Pancreatic enzymes degrade haptocorrin. Cobalamin then binds to intrinsic factor secreted by the pancreas in cats and the stomach and pancreas in dogs.
   e. Cobalamin is absorbed in the ileum where cubilin, a specific receptor for the vitamin-intrinsic factor complex, is present on enterocytes.
   f. Because cobalamin deficiency indirectly inhibits nucleic acid synthesis and thus can affect rapidly dividing cell populations, marked hypocobalaminemia can result in intestinal crypt atrophy and malabsorption.
2. Alterations of cobalamin concentration in disease (Case 15)
   a. Hypocobalaminemia occurs in the following:
      (1) Severe small intestinal disease (e.g., inflammatory bowel disease) reduces cobalamin absorption (intestinal disease limited to the ileum is uncommon).
      (2) SIBO results in the binding of cobalamin and the prevention of its absorption.
      (3) With exocrine pancreatic insufficiency, the lack of pancreatic enzymes results in a failure to liberate cobalamin from haptocorrin, decreased intrinsic factor production, and secondary SIBO.
      (4) Surgical removal of the ileum precludes cobalamin absorption.
      (5) Inherited absorptive defects prevent the uptake of cobalamin (e.g., cobalamin deficiency in Giant Schnauzers).
      (6) Hypocobalaminemia is fairly common in cats with gastrointestinal disease.
   b. Hypercobalaminemia occurs with vitamin supplementation.
   c. Cobalamin testing, like serum folate, is not considered a sensitive test for intestinal disease.

F. Serum folate (Table 8.2)
1. Normal absorption
   a. Folate is readily available in the diet of dogs; dietary deficiency is unlikely.
   b. Specific carriers necessary for absorption of folate are present only in the proximal small intestine.
   c. Deconjugation of dietary folate to the monoglutamate form is required before it is absorbed.

www.Ebook777.com

2. Alteration of folate concentration in disease (Case 15)
   a. Decreased serum folate concentration occurs in the following:
      (1) Diseases of the proximal small intestine
      (2) Administration of certain drugs (e.g., phenytoin, sulfasalazine)
      (3) Decreased serum concentration attributable to malabsorption indicates severe disease and depletion of body stores.
   b. Increased serum folate concentrations occur in the following:
      (1) Bacterial overgrowth in which bacteria synthesize folate
      (2) EPI
         (a) Increased folate with EPI suggests SIBO.
         (b) Because folate absorption is enhanced by reductions in intraluminal pH, impaired pancreatic bicarbonate secretion that can occur with EPI may result in increased folate absorption.
      (3) Decreased folate utilization and subsequent increased serum folate concentration can occur secondary to severe hypocobalaminemia. Cobalamin is required for conversion of methylfolate to tetrahydrofolate, the active form necessary for DNA synthesis. Increased serum folate has been observed in cats with severe hypocobalaminemia.
      (4) Irish setters with gluten-sensitive enteropathy reportedly can have increased or decreased serum folate concentration.

G. Utility of serum folate and cobalamin measurement are improved when they are determined concurrently.
   1. Serum folate concentration is commonly increased with EPI and bacterial overgrowth, whereas serum cobalamin concentration is decreased. Bacterial overgrowth results in increased folate production and absorption in the proximal intestine and binding or increased utilization of cobalamin and the prevention of its absorption in the distal small intestine.
   2. Serum cobalamin and folate measurement, however, are not considered sensitive tests for SIBO; one or both tests are frequently normal, even though bacterial overgrowth exists.
   3. Serum folate and cobalamin concentrations are both decreased in severe, diffuse small intestinal disease and sometimes in EPI.

H. Serum unconjugated cholic acid (SUCA; also referred to as serum total unconjugated bile acid, or TUBA) is another indirect test for SIBO.
   1. Intestinal bacteria deconjugate bile acids, resulting in increased intestinal mucosal absorption via passive diffusion and subsequent increased serum concentration of unconjugated cholic acid.
   2. Total bile acid concentration is unchanged.
   3. SUCA testing currently requires a 12-hour fasted blood sample.
   4. Conflicting findings of studies necessitate further investigation to determine the utility of SUCA testing.

II. LYMPHANGIECTASIA

A. Lymphangiectasia is obstruction and dysfunction of the intestinal lymphatic system resulting in protein-losing enteropathy (PLE) and malabsorption.

B. Potential causes include inflammatory or neoplastic infiltration, fibrosis, inadequate or obstructed flow of the thoracic duct, congestive heart failure, pericarditis, or congenital lymphatic malformation. Inflammatory disease is lymphangiectasia's most common cause.

C. Definitive diagnosis requires histologic examination of biopsy specimens.

D. Clinical pathologic changes that can be observed include panhypoproteinemia, lymphopenia, hypocholesterolemia, and hypocalcemia.

Free ebooks ==> www.Ebook777.com

E. Lymphopenia with lymphangiectasia is primarily attributable to loss of lymphocytes in lymphatic fluid, though endogenously produced corticosteroids in response to the stress of illness can also lead to lymphopenia (as well as eosinopenia and mature neutrophilia).

LABORATORY DETECTION OF OTHER DIGESTIVE DISORDERS

I. MEGAESOPHAGUS

A. Megaesophagus is generalized dilation and inadequate peristalsis of the esophagus. Although it can be congenital, it most commonly is an acquired dysfunction. This disease is uncommon in dogs and rare in cats.

B. Definitive diagnosis of megaesophagus is typically made by radiography or fluoroscopic studies.

C. Studies suggest that megaesophagus results from an afferent neural pathway abnormality.

D. Acquired disease is most commonly idiopathic, although megaesophagus can be observed with a number of conditions. When possible, evaluation for primary disease is exhorted for the sake of bringing to light what may be potentially treatable.

E. Broad categories of conditions that can be associated with megaesophagus include neuromuscular disease (e.g., myasthenia gravis, brainstem injury, or neoplasia), endocrinopathies (e.g., hypothyroidism, hypoadrenocorticism), inflammatory and immune-mediated disease (e.g., polymyositis, systemic lupus erythematosis), toxins (e.g., lead, organophosphates), and infection (e.g., *Clostridium botulinum*).

1. Myasthenia gravis
   - **a.** Myasthenia gravis is a congenital or acquired neuromuscular disorder in which there is a reduced number of acetylcholine receptor (AChR) ion channels on the postsynaptic sarcolemmal membrane. Acquired disease results from the deposition of autoantibodies on the subunits of the AChR.
   - **b.** This disorder results in variable degrees of muscular weakness; regurgitation secondary to megaesophagus can be its only presenting clinical sign (focal myasthenia gravis).
   - **c.** Golden Retrievers and German Shepherds are the most commonly affected breeds; however, the disease can occur in any dog.
   - **d.** Myasthenia gravis is the second most common cause of acquired megaesophagus in dogs.
   - **e.** Measurement of AChR antibody titers, a definitive test for myasthenia gravis, is recommended in all adult dogs with megaesophagus.
   - **f.** Dogs diagnosed with myasthenia gravis also should be tested for hypothyroidism; 20% of dogs with myasthenia gravis are also hypothyroid.
2. Endocrinopathies are uncommon causes of megaesophagus in dogs.
   - **a.** Hypothyroidism
     - **(1)** Few dogs with hypothyroidism develop megaesophagus.
     - **(2)** Ideal laboratory evaluation for hypothyroidism includes measurements of total serum thyroxine (TT4), free serum thyroxine (fT4) by equilibrium dialysis, and thyroid stimulating hormone (TSH).
       - ***(a)*** Mistaken diagnosis of hypothyroidism is commonly made by evaluation of only TT4.
       - ***(b)*** Consequences of megaesophagus, primarily pneumonia, predispose a "euthyroid sick" state in which thyroid function is adequate yet TT4 is below the reference interval. fT4 measurement evinces euthyroidism in these cases.
     - **(3)** Megaesophagus in dogs with hypothyroidism rarely resolves with adequate thyroid hormone supplementation.
   - **b.** Hypoadrenocorticism

www.Ebook777.com

(1) Hypoadrenocorticism can be infrequently associated with megaesophagus. Muscle weakness may result from inadequate cortisol and/or the effects of electrolyte abnormalities on membrane potential and neuromuscular function.
(2) Adrenocorticotropic stimulation testing (ACTH stimulation testing) can establish the absence of adequate cortisol production.
(3) Although 90% of dogs with hypoadrenocorticism have hyponatremia and/or hyperkalemia, this combination is not pathognomonic for hypoadrenocorticism.
(4) Megaesophagus secondary to hypoadrenocorticism typically resolves with adequate adrenal hormone supplementation.

F. Additional routine laboratory testing for animals with megaesophagus
1. A complete blood count may reveal a leukocytosis characterized by a neutrophilia and possibly a left shift if aspiration pneumonia is present.
2. A biochemical profile including sodium and potassium measurement, aspartate transferase (AST), and creatine kinase (CK) aid in evaluating for hypoadrenocorticism and muscular inflammatory conditions.

II. PROTEIN-LOSING ENTEROPATHY (PLE)

A. A certain quantity of plasma proteins enters the gastrointestinal tract each day in healthy animals. These proteins are digested into constituent amino acids, which are almost completely reabsorbed and resynthesized into protein by various cells of the body.

B. Excessive intestinal protein loss may be associated with a number of processes involving mucosal ulceration, inflammation, infiltration, congestion, bleeding, and abnormal intestinal lymphatic drainage.
1. Inflammatory bowel disease (lymphoplasmacytic enteritis), alimentary lymphoma, lymphangiectasia, and chronic parasitism (e.g., giardiasis) are among the most common causes of PLE in adult dogs.
2. Hookworm infestation and chronic intussusception (usually observed in young dogs), histoplasmosis, and pythiosis (most commonly seen in the Gulf Coast area) also are causes of PLE.
3. PLE in cats is uncommon; intestinal lymphoma and severe inflammatory bowel disease are the most common pathologies associated with PLE in cats.

C. Hypoproteinemia may occur with excessive enteric protein loss but is nonspecific.
1. Panhypoproteinemia associated only with clinical signs of enteric disease occurs with severe late-stage enteric protein loss that has exceeded increased compensatory synthesis of plasma protein (Case 16, Chapter 6).
2. Associated clinical signs (e.g., diarrhea and weight loss in dogs) of enteric disease are usually present with PLE.
3. Other possible causes of hypoproteinemia (renal disease, liver disease, malnutrition, hemorrhage, etc.) must be excluded.
a. Protein-losing nephropathy causes decreased serum albumin and an increased urine protein:creatinine ratio; globulin concentration remains unchanged.
b. Liver insufficiency/failure results in decreased serum albumin and increased postprandial bile acids concentration.

D. Fecal α_1-proteinase inhibitor (α_1-PI) immunoassay
1. α_1-PI, a plasma glycoprotein with a molecular weight similar to that of albumin, is present in plasma, lymph, and the intercellular space. It is not present in the lumen of the gastrointestinal tract above trace background concentrations.

Free ebooks ==> www.Ebook777.com

2. α_1-PI may reach the intestinal lumen if there is transmucosal loss of plasma, blood, lymph, or intercellular fluid as a result of gastrointestinal disease. Synthesis of α_1-PI by enterocytes in dogs with intestinal disease has not been critically evaluated.
3. α_1-PI is not degraded in the intestinal lumen.
4. In contrast to identification of panhypoproteinemia, fecal α_1-PI is a better marker for early detection of PLE since it has been shown to be elevated in cases of increased intestinal protein loss prior to the development of hypoproteinemia.
5. Elevated fecal α_1-PI signals the need for intestinal biopsy to determine a specific cause for PLE.
6. Assays for adult dogs and cats are currently available at the Gastrointestinal Laboratory at Texas A & M University, College of Veterinary Medicine, College Station, TX 77843; (979) 862–2861 (www.cvm.tamu.edu/gilab/).

E. Quantitation of radiolabeled protein loss into the intestinal tract
1. ^{51}Cr-labled ceruloplasmin and ^{51}Cr-labeled albumin traditionally have been used to diagnose PLE in the dog.
2. Proper handling of radioactive material and the need for fecal collection for three days limit the usefulness of this methodology.

F. Hypocalcemia
1. Hypocalcemia in dogs with PLE is not uncommon and is typically associated with hypoalbuminemia. Two studies have shown that after correction for decreased serum albumin concentration, total calcium concentration was within the reference interval for the vast majority of dogs.
2. Rare cases of ionized hypocalcemia associated with decreased serum 25-hydroxyvitamin D and increased parathyroid hormone concentrations have been reported in dogs with PLE.
a. These findings suggest that PLE can result in compromised vitamin D absorption and secondary hyperparathyroidism.
b. Studies suggest that lymphangiectasia may be more commonly associated with ionized hypocalcemia than other causes of PLE.

III. EQUINE HYPERAMMONEMIA

A. Hyperammonemia and subsequent encephalopathy in horses can occur with liver dysfunction, congenital enzyme deficiencies or abnormal transport in the urea cycle, and gastrointestinal disease (colic).

B. Proposed mechanisms for the development of hyperammonemia in horses with colic without hepatic disease are increased absorption of ammonia across an inflamed intestinal mucosa, marked overproduction of ammonia by urease-producing bacteria within the intestine, or both.
1. Ultimately, the urea cycle within hepatocytes is overwhelmed by the amount of ammonia delivered by enterohepatic circulation.
2. Urease-producing bacteria include Gram-negative bacilli (e.g., *Escherichia coli*, *Klebsiella*, *Proteus*, and *Pseudomonas* spp.) and Gram-positive bacilli (namely subtypes of *Clostridium* spp.).

C. Definitive diagnosis of hyperammonemia secondary to gastrointestinal disease requires detection of increased blood ammonia levels and exclusion of hepatic dysfunction, abnormal portal circulation, or urea/ammonia intoxication.
1. The severe self-destructive behavior typically associated with hyperammonemic encephalopathy can necessitate euthanasia prior to diagnosis.

www.Ebook777.com

2. A recent report of a horse with peracute colitis demonstrated that an antemortem presumptive diagnosis of hyperammonemia could be confirmed by measurement of ammonia in cerebrospinal and intraocular (aqueous humor) fluids collected postmortem.

IV. CYTOLOGY

A. Cytology can aid in the diagnosis of inflammatory, infectious, and neoplastic disorders associated with large bowel diarrhea via evaluation of feces (primarily the stool "surface") or rectal scrapings.

1. Intact neutrophils in Wright-stained fecal smears indicate inflammation of the lower small intestine and/or colon.

2. Pathogenic organisms may be found in cytologic preparations of rectal scrapings (e.g., *Mycobacterium paratuberculosis, Histoplasma capsulatum, Prototheca zopfii*).

3. Although *Campylobacter* sp. are classically described as "gull-shaped" organisms, they are not readily differentiated cytologically from nonpathogenic spirochetes. If campylobacteriosis is suspected, fecal culture is recommended for confirmation.

4. The presence of safety-pin-shaped Gram-positive bacilli (*Clostridium* sp. spores) may be supportive of but not definitive for diagnosis of clostridial enterotoxicosis.

B. An increased nucleated cell count and/or protein concentration in abdominal effusions are found in a variety of conditions associated with equine colic (Chapter 12).

REFERENCES

Banks PA: 1998. Acute and Chronic Pancreatitis. *In:* Feldman M, Scharschmidt BF, Sleisenger MH (eds): *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*, 6th ed. Philadelphia, WB Saunders, pp. 809–862.

Batt RM: 1982. Role of serum folate and vitamin B_{12} concentrations in the differentiation of small intestinal abnormalities in the dog. *Res Vet Sci* 32:17–22.

Batt RM: 1983. Bacterial overgrowth associated with a naturally occurring enteropathy in the German shepherd dog. *Research Vet Sci* 35:42–46.

Batt RM: 1986. New approaches to malabsorption in dogs. *Compend Contin Educ Pract Vet* 8:783–794.

Batt RM: 1993. Exocrine pancreatic insufficiency. *Vet Clin North Am Small Anim Pract* 23:595–608.

Brenner K, Harkin KR, Andrews GA, et al: 2009. Juvenile pancreatic atrophy in greyhounds: 12 cases (1995–2000). *J Vet Intern Med* 23:67–71.

Brobst DF: 1997. Pancreatic Function. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 5th ed. San Diego, Academic Press, pp. 353–366.

Brown CM: 1992. The diagnostic value of the d-xylose absorption test in horses with unexplained chronic weight loss. *Br Vet J* 148:41–44.

Desrochers AM, Dallap BL, Wilkins PA: 2003. *Clostridium sordelli* infection as a suspected cause of transient hyperammonemia in an adult horse. *J Vet Intern Med* 17:238–241.

Dutta SK, Russell RM, Iber FL: 1979. Influence of exocrine pancreatic insufficiency on the intraluminal pH of the proximal small intestine. *Am J Digestive Disease* 24:529–534.

Diehl KJ, Lappin MR, Jones RL, et al: 1993. Monoclonal gammopathy in a dog with plasmacytic gastroenterocolitis. *J Am Vet Med Assoc* 201:1233–1236.

Free ebooks ==> www.Ebook777.com

Doneley RJ: 2001. Acute pancreatitis in parrots. *Aust Vet J* 79:409–411.

Dupuy B, Daube G, Popoff MR, et al: 1997. *Clostridium perfringens* urease genes are plasmid borne. *Infect Immun* 65:2313–2320.

Edwards DF, Bauer MS, Walker MA, et al: 1990. Pancreatic masses in seven dogs following acute pancreatitis. *J Am Anim Hosp Assoc* 26:189–198.

Edwards DF, Russell RG: 1987. Probable vitamin-K-deficient bleeding in two cats with malabsorption syndrome secondary to lymphocytic-plasmacytic enteritis. *J Vet Intern Med* 1:97–101.

Ferreri JA, Hardam E, Kimmel SE, et al: 2003. Clinical differentiation of acute necrotizing from chronic nonsuppurative pancreatitis in cats: 63 cases (1996–2001). *J Am Vet Med Assoc* 223:469–474.

Forcada Y, German AJ, Noble PJM, et al: 2008. Determination of serum fPLI concentrations in cats with diabetes mellitus. *J Feline Med Surg* 10:480–487.

Foreman MA, Marks SL, DeCook HEV, et al: 2004. Evaluation of serum feline pancreatic lipase immunoreactivity and helical computed tomography versus conventional testing for the diagnosis of feline pancreatitis. *J Vet Intern Med* 18:807–815.

Fossum TW: 1989. Protein-losing enteropathy. *Semin Vet Med Surg (Small Anim)* 4;219–225.

Gerhardt A, Steiner JM, Williams DA, et al: 2001. Comparison of the sensitivity of different diagnostic tests for pancreatitis in cats. *J Vet Intern Med* 15:329–333.

German AJ, Day MJ, Ruaux CG, et al: 2003. Comparison of direct and indirect tests for small intestinal bacterial overgrowth and antibiotic-responsive diarrhea in dogs. *J Vet Intern Med* 17:33–43.

German AJ, Hall EJ, Day MJ: 2003. Chronic intestinal inflammation and intestinal disease in dogs. *J Vet Intern Med* 17:8–20.

Gilliam LL, Holbrook TC, Dechant JE, et al: 2007. Postmortem diagnosis of idiopathic hyperammonemia in a horse. *Vet Clin Pathol* 36:196–199.

Hall EJ, Batt RM: 1990. Enhanced intestinal permeability to ^{51}Cr-labeled EDTA in dogs with small intestinal disease. *J Am Vet Med Assoc* 196:91–95.

Hall E, Simpson K: 2000. Diseases of the Small Intestine. *In:* Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine: Disease of the Cat and Dog*, 5th ed. WB Saunders, Philadelphia, pp. 1182–1237.

Hall JA, Macy DW: 1988. Acute canine pancreatitis. *Compend Contin Educ Pract Vet* 10:403–416.

Hill PG: 2001. Fecal fat: Time to give it up. *Ann Clin Biochem* 38:164–167.

Hoffman WE: 2008. Diagnostic Enzymology of Domestic Animals. *In*: Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 6th ed. Elsevier, Boston, pp. 351–378.

Holm JL, Rozanski EL, Freeman LM, et al: 2004. C-reactive protein concentrations in acute pancreatitis. *J Vet Em Crit Care* 14:183–186.

Hornbuckle WE, Simpson KW, Tennant BC: 2008. Gastrointestinal Function. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 6th ed. Elsevier, Boston, pp. 413–458.

Hornbuckle WE, Tennant BC: 1997. Gastrointestinal Function. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 5th ed. Academic Press, San Diego, pp. 367–406.

House JK, Smith BP, VanMetre DC, et al: 1992. Ancillary tests for assessment of the ruminant digestive system. *Vet Clin North Am Food Anim Pract* 8:203–232.

www.Ebook777.com

Hurley PR, Cook A, Jehanli A, et al: 1988. Development of radioimmunoassay for free tetra-l-aspartyl-l-aspartyl-l-lysine trypsinogen activation peptides (TAP). *J Immunol Methods* 111:195–203.

Jacobs RM: 1988. Renal disposition of amylase, lipase, and lysosome in the dog. *Vet Pathol* 25:443–449.

Jacobs RM: 1989. The origins of canine serum amylase and lipase. *Vet Pathol* 26:525–527.

Jacobs RM, Norris AM, Lumsden JH, et al: 1989. Laboratory diagnosis of malassimilation. *Vet Clin North Am Small Anim Pract* 19:951–977.

Jergens AE, Moore FM, Haynes JS, et al: 1992. Idiopathic inflammatory bowel disease in dogs and cats: 84 cases (1987–1990). *J Am Vet Med Assoc* 201:1603–1608.

Johnston KL: 1999. Small intestinal bacterial overgrowth. *Vet Clin North Am Small Anim Pract* 29:523–550.

Khouri MR, Huang G, Shiau YF: 1989. Sudan stain of fecal fat: New insight into an old test. *Gastroenterology* 96:421–427.

Kimmel SE, Waddell LS, Michel KE: 2000. Hypomagnesemia and hypocalcemia associated with protein losing enteropathy in Yorkshire Terriers: Five cases (1992–1998). *J Am Vet Med Assoc* 217:703–706.

Kimmel SE, Washabau RJ, Drobatz KJ: 2001. Incidence and prognostic value of low plasma ionized calcium concentration in cats with acute pancreatitis: 46 cases (1996–1998). *J Am Vet Med Assoc* 219:1105–1109.

Ludlow CL, Davenport DJ: 2000. Small Intestinal Bacterial Overgrowth. *In:* Bonagura JD (ed): *Current Veterinary Therapy XIII*. WB Saunders, Philadelphia, pp. 637–641.

MacAllister CG, Mosier D, Qualls CW, et al: 1990. Lymphocytic-plasmacytic enteritis in two horses. *J Am Vet Med Assoc* 196:1995–1998.

Macy DW: 1989. Feline Pancreatitis. *In:* Kirk RW (ed): *Current Veterinary Therapy X: Small Animal Practice*. WB Saunders, Philadelphia, pp. 893–896.

Mansfield CS, Jones BR: 2000. Trypsinogen activation peptide in the diagnosis of canine pancreatitis. *J Vet Intern Med* 14:346 (ACVIM abstract #75).

Mansfield CS, Jones BR: 2000. Plasma and urinary trypsinogen activation peptide in healthy dogs, dogs with pancreatitis and dogs with other systemic diseases. *Aust Vet J* 78:416–422.

Mansfield CS, Jones BR: 2001. Review of feline pancreatitis part two: Clinical signs, diagnosis and treatment. *J Feline Med Surg* 3:125–132.

Marks SL. 2003. Editorial: Small intestinal bacterial overgrowth in dogs—Less common than you think? *J Vet Intern Med* 17:5–7.

Matz ME, Guilford WG: 2003. Laboratory procedures for the diagnosis of gastrointestinal diseases of dogs and cats. *N Z Vet J* 51:292–301.

Melgarejo T, Williams DA, Asem EK: 1998. Enzyme-linked immunosorbent assay for canine alpha 1-protease inhibitor. *Am J Vet Res* 59: 59:127–30 and 524 (erratum).

Melgarejo T, Williams DA, O'Connell NC, et al: 2000. Serum unconjugated bile acids as a test for intestinal bacterial overgrowth. *Digest Dis Sci* 45:407–414.

Mellanby RJ, Mellor PJ, Roulois A, et al: 2005. Hypocalcaemia associated with low serum vitamin D metabolite concentrations in two dogs with protein-losing enteropathies. *J Small Anim Pract* 46:345–351.

Free ebooks ==> www.Ebook777.com

Munro DR: 1974. Route of protein loss during a model protein-losing gastropathy in dogs. *Gastroenterology* 66:960–972.

Murphy KF, German AJ, Ruaux CG, et al: 2003. Fecal α_1 proteinase inhibitor concentration in dogs with chronic gastrointestinal disease. *Vet Clin Pathol* 32:67–72.

Nakamura M, Takahashi M, Ohno K, et al: 2008. C-reactive protein concentration in dogs with various diseases. *J Vet Med Sci* 70:127–131.

Nix BE, Leib MS, Zajac A, et al: 1993. The effect of dose and concentration on d-xylose absorption in healthy, immature dogs. *Vet Clin Pathol* 22:10–16.

Olchowy TWJ, Linnabary RD, Andrews FM, Longshore RC: 1993. Lactose intolerance in a calf. *J Vet Intern Med* 7:12–15.

Oran DL: 2006. Pancreatitis in cats: Diagnosis and management of a challenging disease. *J Am Anim Hosp Assoc* 42:1–9.

Panagiotis GX, Steiner JM: 2008. Current concepts in feline pancreatitis. *Top Companion Anim Med* 23:185–192.

Papasouliotis K, Sparkes AH, Gruffydd-Jones TJ, et al: 1998. Use of the breath hydrogen test to assess the effect of age on orocecal transit time and carbohydrate assimilation in cats. *Am J Vet Res* 59:1299–1302.

Quigley KA, Jackson ML, Haines DM: 2001. Hyperlipasemia in 6 dogs with pancreatic or hepatic neoplasia: Evidence for tumor lipase production. *Vet Clin Pathol* 30:114–120.

Reed N, Gunn-Moore D, Simpson K: 2007. Cobalamin, folate, and inorganic phosphate abnormalities in ill cats. *J Feline Med Surg* 9:278–288.

Rinderknect H: 1986. Activation of pancreatic zymogens: normal activation, premature intrapancreatic activation, protective mechanisms against inappropriate activation. *Dis Sci* 31:314–321.

Roberts MC: 1985. Malabsorption syndromes in the horse. *Compend Contin Educ Pract Vet* 7:S637–S646.

Ruaux CG, Steiner JM, Williams DA: 2003. Protein-losing enteropathy in dogs is associated with decreased fecal proteolytic activity. *Vet Clin Pathol* 33:20–22.

Ruaux CG, Steiner JM, Williams DA: 2005. Early biochemical and clinical responses to cobalamin supplementation in cats with signs of gastrointestinal disease and severe hypocobalaminemia. *J Vet Intern Med* 19:155–160.

Rucker RB, Morris JG, Fascetti AJ: 2008. Vitamins. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 6th ed. Elsevier, Boston, pp. 695–730.

Russell RM, Dhar GJ, Dutta SK, et al: 1979. Influence of intraluminal pH on folate absorption: Studies in control subjects and in patients with pancreatic insufficiency. *J Lab Clin Med* 93:428–436.

Rutgers HC, Batt RM, Kelly DF: 1988. Lymphocytic-plasmacytic enteritis associated with bacterial overgrowth in a dog. *J Am Vet Med Assoc* 192:1739–1742.

Sharkey LC, DeWitt S, Stockman C: 2006. Neurologic signs and hyperammonemia in a horse with colic. *Vet Clin Pathol* 35:254–258.

Sherding RG: 2003, Diseases of the Large Intestine. In: Tams TR (ed): *Handbook of Small Animal Gastroenterology*, 2nd ed. Elsevier Science, St. Louis, pp. 251–285.

Simpson KW: 2003. Diseases of the Pancreas. In: Tams TR (ed): *Handbook of Small Animal Gastroenterology*, 2nd ed. Elsevier Science, St. Louis, pp. 353–369.

www.Ebook777.com

Simpson JW, Doxey DL: 1988. Evaluation of faecal analysis as an aid to the detection of exocrine pancreatic insufficiency. *Br Vet J* 144:174–178.

Simpson KW, Fyfe J, Cornetta A, et al: 2001. Subnormal concentrations of serum cobalamin (vitamin B_{12}) in cats with gastrointestinal disease. *J Vet Intern Med* 15:26–32.

Sorensen SH, Proud FJ, Adam A, et al: 1993. A novel HPLC method for the simultaneous quantification of monosaccharides and disaccharides used in tests of intestinal function and permeability. *Clinica Chimica Acta* 221:115–125.

Steiner JM: 2003. Diagnosis of pancreatitis. *Vet Clin North Am Small Anim Pract* 33:1181–1195.

Steiner JM: 2010. Is it pancreatitis? Veterinary Medicine, www.veterinarymedicine.dvm360.com, 2006, visited 10 Mar 2010.

Steiner JM, Broussard J, Mansfield CS, et al: 2001. Serum canine pancreatic lipase immunoreactivity (cPLI) concentrations in dogs with spontaneous pancreatitis. *J Vet Intern Med* 15:274.

Steiner JM, Finco DR, Gumminger SR, et al: 2001. Serum canine pancreatic lipase immunoreactivity (cPLI) in dogs with experimentally induced chronic renal failure (abstract). *J Vet Intern Med* 15:311.

Steiner JM, Lees GE, Willard MD, et al: 2003. Serum canine pancreatic lipase immunoreactivity (cPLI) concentration is not altered by oral prednisone (abstract). *J Vet Intern Med* 17:444.

Steiner JM, Rutz GM, Williams DA.: 2006. Serum lipase activities and pancreatic lipase immunoreactivity concentrations in dogs with exocrine pancreatic insufficiency. *Am J Vet Res* 67:84–77.

Steiner JM, Williams DA: 2000. Serum feline trypsin-like immunoreactivity in cats with exocrine pancreatic insufficiency. *J Vet Intern Med* 14: 627–629.

Steiner JM, Williams DA: 1997. Feline exocrine pancreatic disorders: Insufficiency, neoplasia and uncommon conditions. *Compend Contin Educ Vet* 19:836–848.

Steiner JM, Williams DA: 2000. Serum feline trypsin-like immunoreactivity in cats with exocrine pancreatic insufficiency. *J Vet Intern Med* 14:627–629.

Steiner JM, Williams DA: 1999. Feline exocrine pancreatic disorders. *Vet Clin North Am Small Anim Pract* 29:551–575.

Strombeck DR, Harrold D: 1982. Evaluation of 60-minute blood p-aminobenzoic acid concentration in pancreatic function testing of dogs. *J Am Vet Med Assoc* 180:419–421.

Suchodolski JS, Ruaux CG, Steiner JM, et al: 2001. Serum alpha 1-proteinase inhibitor/trypsin complex as a marker for canine pancreatitis. *J Vet Intern Med* 15:273 (ACVIM abstract #8).

Swift NC, Marks SL, MacLachlan NJ, et al: 2000. Evaluation of serum feline trypsin-like immunoreactivity for the diagnosis of pancreatitis in cats. *J Am Vet Med Assoc* 217:37–42 and 816–818(comment).

Tams TR: 2003.Diseases of the Esophagus. *In*: Tams TR (ed): *Handbook of Small Animal Gastroenterology*, 2nd ed. Elsevier Science, St. Louis, pp. 118–158 and 211–250.

Texas A & M Website: 2010. http://www.cvm.tamu.edu/gilab/research/Pancreatitis.shtml, visited 10 March 2010.

Thompson KA, Parnell NK, Hohenhaus AE, et al: 2009. Feline exocrine pancreatic insufficiency: 16 cases (1992–2007). *J Feline Med Surg* 11:935–940.

Triolo A, Lappin MR: 2003. Acute Medical Diseases of the Small Intestine. *In:* Tams TR (ed): *Handbook of Small Animal Gastroenterology*, 2nd ed. Elsevier Science, St. Louis, pp.195–210.

Free ebooks ==> www.Ebook777.com

Ugarte CE: 2003. The role of diet in feline inflammatory bowel disease. PhD dissertation, Massey University, Palmerston North.

Washabau RJ, Strombeck DR, Buffington CA, et al: 1986. Evaluation of intestinal carbohydrate malabsorption in the dog by pulmonary hydrogen gas excretion. *Am J Vet Res* 40:1201–1206.

Washabau RJ, Strombeck DR, Buffington CA, et al: 1986. Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. *J Am Vet Med Assoc* 189:674–679.

Watson PJ: 2003. Exocrine pancreatic insufficiency as an end stage of pancreatitis in four dogs. *J Small Anim Pract* 44:306–312.

Westermarck E: 1980. The hereditary nature of canine pancreatic degenerative atrophy in German shepherd dogs. *Acta Vet Scand* 21:389–394.

Westermarck E, Pamilo P, Wiberg M: 1989. Pancreatic degenerative atrophy in the collie breed: A hereditary disease. *J Vet Med Assoc* 36:549–554.

Weiss DJ, Gagne JM, Armstrong PJ: 1996. Relationship between inflammatory hepatic disease and inflammatory bowel disease, pancreatitis, and nephritis in cats. *J Am Vet Med Assoc* 209:1114–1116.

Wiberg ME, Nurmi A-K, Westermarck E: 1999. Serum trypsin-like immunoreactivity measurement for the diagnosis of subclinical exocrine pancreatic insufficiency in dogs. *J Vet Intern Med* 13:426–432.

Wiberg ME, Westermarck E. 2002. Subclinical exocrine pancreatic insufficiency in dogs. *J Am Vet Med Assoc* 220:1183–1187.

Williams DA: 1996. The Pancreas. *In*: Strombeck DR, Williams DA, Meyer DJ, Guilford WE, Center SA (eds): *Strombeck's Small Animal Gastroenterology*, 3rd ed. WB Saunders, Philadelphia, pp. 381–410.

Williams DA, Guilford WG: 1996. Procedures for the Evaluation of Pancreatic and Gastrointestinal Tract Diseases. *In:* Guilford WG, Center SA, Strombeck DR, Williams DA, Meyer DJ (eds): *Strombeck's Small Animal Gastroenterology*, 3rd ed. WB Saunders, Philadelphia, pp. 77–113.

Williams DA, Batt RM, McLean L: 1987. Bacterial overgrowth in the duodenum of dogs with exocrine pancreatic insufficiency. *J Am Vet Med Assoc* 191:201–206.

Williams DA, Minnich F: 1990. Canine exocrine pancreatic insufficiency—A survey of 640 cases diagnosed by assay of serum trypsin-like immunoreactivity. *J Vet Intern Med* 4:123.

Williams DA, Reed SD: 1990. Comparison of methods for assay of fecal proteolytic activity. *Vet Clin Pathol* 19:20–24.

Williams DA, Reed SD, Perry L: 1990. Fecal proteolytic activity in clinically normal cats and in a cat with exocrine pancreatic insufficiency. *J Am Vet Med Assoc* 197:210–212.

Xenoulis PG, Steiner JM: 2008. Current concepts in feline pancreatitis. *Top Companion Anim Med* 23:185–192.

Zoran DL: 2006. Pancreatitis in cats: Diagnosis and management of a challenging disease. *J Am Animal Hosp Assoc* 42:1–9.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 9

URINARY SYSTEM

Niraj K. Tripathi, BVScAH, MVSc, PhD; Christopher R. Gregory, DVM, PhD; and Kenneth S. Latimer, DVM, PhD

URINALYSIS

Examination of urine is a very useful procedure to evaluate renal function in animals. Urinalysis not only assesses renal function but also may reflect a variety of systemic disease processes (e.g., inflammation, hemorrhage, and intravascular hemolysis). Because birds have a cloaca that pools both urinary and intestinal wastes, urinalysis is not as useful in birds as it is in mammals.

I. METHODS OF COLLECTION

A. Voided specimens

1. Contamination of urine from the lower urinary tract and reproductive tract may occur in mammals. Common contaminants include bacteria, leukocytes, spermatozoa, and epithelial cells.

2. Midstream ("clean catch") collection of urine may reduce contamination. This is the preferred method of urine collection in large animals.

3. Birds lack a urinary bladder. Urine is discharged through the cloaca, a common opening for the urinary, digestive, and reproductive tracts of birds.

a. Contamination of avian urine is common and usually obscures evaluation of its physical, chemical, and microscopic characteristics.

b. Urine contains a solid component of urates, a pasty white to yellow substance. Urates may impede detailed microscopic examination of avian urine. Several drops of sodium hydroxide may be added to a wet mount of avian urine to dissolve urates; however, this basic solution also may dissolve casts.

c. Excited or stressed birds often are polyuric, enabling collection of the liquid component of urine.

B. Catheterized specimens

1. Care must be taken to clean the urethral orifice and surrounding area to reduce contamination of the urine specimens and prevent the introduction of pathogens into the urinary tract.

2. Traumatic catheterization may dislodge transitional epithelial cells from the urethra and cause iatrogenic hemorrhage.

3. Ureteral catheterization has been reported in pigeons but is not commonly used in clinical practice.

www.Ebook777.com

C. Cystocentesis specimens

1. Cystocentesis is the preferred method of urine collection in small mammals such as dogs and cats.

2. Potential problems include iatrogenic hemorrhage or unanticipated enterocentesis with subsequent contamination of the urine specimen.

II. PROPER SAMPLE HANDLING

A. The urine specimen should be collected prior to medical therapy or contrast radiography, if possible.

B. Collection vessels should be clean, detergent and reagent free, and sterile if microbiological culture is indicated.

C. Ideally, the urinalysis should be performed within 30 minutes of specimen collection.

1. The urine specimen may be refrigerated, but not frozen, up to 12 hours if urinalysis is delayed.

2. The urine specimen should be re-warmed to room temperature before analysis.

a. Precipitates that form at lower temperatures will redissolve.

b. Inhibition of enzymatic activities by low temperatures will be avoided.

D. Avian urine

1. The liquid component of "urine" should be collected from an impervious surface as soon as possible after excretion.

III. PHYSICAL CHARACTERISTICS

A. Color

1. Normal mammalian urine is yellow to amber. The depth of color is related to urine volume and concentration. Urochrome and urobilin are responsible for the normal color of urine.

2. Abnormal urine color can obscure some dipstick test results. Causes of abnormal urine color include the following:

a. Blood (hematuria) is red; the urine appears cloudy and usually clears following centrifugation.

b. Bilirubin appears dark yellow to brown.

c. Hemoglobin and myoglobin are red to red-brown.

d. Porphyrins are colorless but produce pink fluorescence in acid urine when exposed to ultraviolet light.

e. Certain drugs and metabolic diseases may alter the color of urine (e.g., brown-black urine after Oxyglobin® administration).

3. Avian urine

a. Urates

(1) Yellow-green urates indicate hemolysis or liver disease (biliverdinuria).

(2) Idiopathic red-brown urates have been described in hand-fed chicks receiving an animal protein-based diet.

b. Diet (e.g., blue-purple urine from fruit), medications, and mixing of urine with feces can change the color of avian urine.

c. Lead toxicosis may result in tan to brown discoloration of urine and urates ("chocolate milk" appearance).

B. Transparency (clarity, turbidity)

1. Normal urine generally is clear when freshly voided, but may become cloudy on standing or refrigeration from precipitation of salts.

2. Fresh normal horse urine is cloudy because of calcium carbonate crystals and mucus (Cases 3, 17).

Free ebooks ==> www.Ebook777.com

3. Cloudy, opaque, or flocculent urine may be seen in some birds, particularly in the orders Struthioniformes (ratites) and Anseriformes (ducks, geese).
4. The cause of cloudy or turbid urine should always be identified microscopically. Causes of cloudiness and turbidity include crystals, cells, mucus, bacteria, casts, and spermatozoa (see Sediment Examination later in this chapter). Many of these causes are not pathologic.

C. Odor
1. Ammonia is formed from urea by bacterial urease action. Ammonia is particularly prominent in retained urine or old urine specimens.
2. An acetone odor suggests ketosis.
3. Excretion of certain drugs may give characteristic odors to the urine.

D. Volume
1. Control of urine volume
a. Urine enters the proximal tubule at approximately the same osmolality as plasma.
b. Obligatory reabsorption of water, independent of body needs, occurs in the proximal tubules. By osmotic action, water follows sodium, glucose, and other solutes that are actively reabsorbed. Urine is iso-osmotic compared to plasma upon entering the loop of Henle.
c. The osmolality of the urine increases in the descending loop of Henle, which is highly permeable to water but rather impermeable to solute.
d. The ascending loop of Henle is permeable to solute but rather impermeable to water. The ascending loop also is the site of active chloride transport. Urine entering the distal tubule is hypo-osmotic compared to plasma.
e. Water reabsorption in the distal and collecting tubules is in excess of solute. It is under the control of antidiuretic hormone (ADH) and requires a hypertonic medulla. The major control of urine volume occurs at this level.
f. Maintenance of medullary hypertonicity is a function of the countercurrent multiplier system of the loop of Henle and vasa recta.
2. Methods of measurement
a. The measurement of the total urine volume that is voided during a 24-hour period is the most accurate measurement of urine output. Such measurement requires a metabolism cage, which is impractical in most clinical settings.
b. Estimation of volume may be inferred from urine specific gravity or osmolality. Volume and specific gravity or osmolality are inversely related in health and in most diseases. Exceptions include the following:
(1) Diabetes mellitus. Polyuria and high specific gravity coexist because of glucosuria. Glucose increases urine specific gravity at the rate of 0.004 units/g/dL of urine glucose.
(2) Acute and chronic renal disease. Oliguria may be accompanied by a lack of renal concentrating ability.
3. Causes of abnormal urine volume are listed in Table 9.1.

E. Solute concentration
1. Methods of measurement
a. Osmolality (Chapter 5)
(1) Measurement of osmolality depends on particle numbers in solution.
(2) Depression of freezing point and lowering of vapor pressure are the methods of measurement.
b. Specific gravity
(1) Specific gravity depends on particle number, size, and weight. Specific gravity is the ratio of the refractive index of urine compared to water.
(2) Specific gravity is easily determined by refractometry and is a valid reflection of osmolality.

www.Ebook777.com

TABLE 9.1
CAUSES OF ABNORMAL URINE VOLUME.

Polyuria	Oliguria
Acute renal disease	Acute renal disease
Chronic renal disease	Dehydration
Diabetes mellitus	Shock
Diets with high moisture content (especially birds)	Terminal chronic renal disease
Hepatic failure	Urinary tract obstruction
Hyperadrenocorticism	
Hypercalcemia	
Hyperparathyroidism (cats)	
Hypoadrenocorticism	
Hypokalemia	
Hyponatremia	
Hypovitaminosis A, hypervitaminosis D_3 (birds)	
Nephrogenic diabetes insipidus	
Pituitary diabetes insipidus	
Postobstructive diuresis	
Primary renal glucosuria	
Psychogenic polydipsia	
Pyelonephritis	
Pyometra	
Stress, excitement (especially birds)	

(3) Refractometry is the easiest method to measure urine specific gravity in clinical practice. Human reagent (dipstick) pads are unreliable in measuring urine specific gravity in animals.

(4) Important considerations in refractometry include the following:

(a) Measurement of refractive index is temperature-dependent. Most hand-held refractometers are temperature-compensated to give accurate readings between 16°C and 38°C (60°F to 100°F).

(b) Veterinary models of the instrument provide more accurate value of specific gravity in concentrated feline urine specimens.

(c) Quality control consists of calibrating the instrument by measuring the specific gravity of water (1.000) and a $5\%^{w/v}$ sodium chloride solution (1.022). Controls with known low and high specific gravities also should be used.

2. Interpretation (also see urine concentration tests)

a. Knowledge of the hydration status of the animal is necessary to interpret the urine specific gravity.

b. Urine specific gravity can range from 1.001 to 1.065 in most healthy animals and up to 1.080 in the cat. This range includes values associated with renal abnormalities. Urine specific gravity ranges from 1.005 to 1.020 in birds.

c. Adequate renal concentrating ability is presumed to exist if the specific gravity of a random urine specimen is: greater than 1.030 in dogs, greater than 1.035 in cats, greater than 1.020 birds, and greater than 1.025 in other species. For exceptions to these generalizations, see interpretation of increased BUN concentration and renal azotemia.

d. Renal failure exists if urine is inadequately concentrated and the animal is azotemic. Renal failure defined in this manner may be primary renal disease or secondary to other diseases (Table 9.1). Depending on the cause, renal failure may be reversible or irreversible.

Free ebooks ==> www.Ebook777.com

e. Isosthenuria ("fixed" specific gravity) is the constant maintenance of urine osmolality in the range of the glomerular filtrate (specific gravity = 1.008 to 1.012). In isosthenuria, the kidney is neither concentrating nor diluting the urine.

f. Hyposthenuria is defined as a urine specific gravity less than 1.008.

(1) In hyposthenuria, the kidney retains some water-balance function whereby solute is being reabsorbed in excess of water (Case 13).

(2) The ability to concentrate urine and preferentially reabsorb solute is lost in polyuric renal disease.

g. Most neonates, except calves, do not have efficient urine concentrating mechanisms.

h. Relative increases of analytes, such as protein and bilirubin, are present in concentrated urine.

i. Erythrocytes may lyse in hypotonic urine (specific gravity less than 1.006); erythrocyte ghosts may be observed in the urine sediment.

j. Glucose and protein may falsely elevate the specific gravity of urine.

k. Urine osmolality occasionally is measured in birds and should be at least 450 mOsmol/kg following 24 hours of water deprivation. Maximum values may range from 500 to 1,000 mOsmol/kg. See Chapter 5 for a general discussion on osmolality.

IV. CHEMICAL CHARACTERISTICS

Various chemical substances (e.g., protein, glucose, ketones, bilirubin, occult blood, and urobilinogen) are measured semiquantitatively in the urine by reagent strips, which have color coded pads with specific reagents for each test. The change of color in these pads indicates the presence or absence of these analytes in the urine and provides a rough estimation of their quantity (semiquantitative). Most commonly available reagent strips are designed for humans but specific test strips for animals are also available. Test results are usually interpreted by visual inspection, but some laboratories with high throughput use automated instruments to interpret the reagent strip test results.

A. Protein

1. Physiology

a. The size, shape, and charge of protein molecules influence their ability to pass the glomerular filter.

b. Low-molecular-weight globulins (MW = 15,000 to 20,000 Da) are freely filtered by the glomerulus, but their small quantity in urine reflects their low concentration in plasma. A small quantity of albumin (MW = 66,000 Da) also is filtered from plasma. Most of these filtered proteins subsequently are reabsorbed by the renal tubules.

c. The small quantity of protein that normally remains in the urine is not detectable by clinical screening tests.

d. Tamm-Horsfall protein and IgA are secreted by the tubules in clinically undetectable amounts.

e. Increased urine protein may cause formation of persistent foam, particularly if the urine is shaken.

(1) Shaking the urine specimen may destroy certain elements in the sediment such as casts.

(2) Normal urine also will foam but the foam is not persistent.

(3) Analytes that impart color to the urine may also color the foam. Bilirubin will stain the foam greenish-yellow to brown, while hemoglobin and myoglobin will stain foam red to red-brown.

2. Methods of measurement

a. Semiquantitation of urine protein is commonly done using reagent strip (dipstick) tests.

(1) This test is based on the "protein error of pH dye indicators." The reagent pad is buffered at a pH of 3. An increase in anions, in this instance negatively-charged proteins, causes a pH indicator dye to shift color from yellow to green to blue.

www.Ebook777.com

(2) The reagent strip method is most sensitive for albumin. It will not reliably detect globulins or Bence-Jones paraprotein of plasma cell myeloma (Chapter 3).

(3) The intensity of the green to blue color is proportional to the protein concentration.

(4) Reagent strips yield negative, trace, and 1 to 4+ reactions. The test results are semiquantitative; exact protein concentrations vary but are usually in the 30 to 2,000 mg/dL range.

(5) If urine pH is alkaline, the reagent pad may change color in the absence of proteinuria.

b. Acid precipitation tests (sulfosalicylic acid, nitric acid, trichloroacetic acid) also provide semiquantitative detection of urine protein.

(1) Acid precipitation detects albumin and non-albumin proteins.

(2) This test differentiates bona fide proteinuria from a false positive dipstick protein reading from alkaline urine.

c. Bence-Jones protein is derived from immunoglobulin light chains and is dissolved in urine at room temperature. When the urine is heated, Bence-Jones protein precipitates at 40°C to 60°C and redissolves at 85°C to 100°C. As the urine is cooled to 40°C–60°C, Bence-Jones protein reprecipitates and then redissolves again as the urine specimen reaches room temperature. However, this temperature-associated precipitation test is not very sensitive in detecting Bence-Jones proteinuria. Immunoelectrophoresis is a more sensitive and specific method of detection.

d. The magnitude of proteinuria should be measured accurately and precisely by quantitative tests to evaluate protein loss. The quantity of protein lost should be evaluated with regard to urine volume or specific gravity. Detection of trace protein loss in a dilute urine (i.e., high urine volume) may be more significant than trace protein loss in concentrated urine (low urine volume). The amount of protein being lost in the urine may be quantified by the following:

(1) Measuring the total amount of protein in a 24-hour collection of urine. This task is difficult in animals. Normal protein excretion is less than 200 mg/day.

(2) Calculating the ratio of urine protein to urine creatinine (UP/UC)

(a) Simultaneous urine protein and urine creatinine determinations are made on a single sample of urine.

(b) This test gives similar information to the 24-hour urine protein determination from a single collection, but may not be equally reliable because it reflects renal function over a relatively short duration compared to a 24-hour collection.

(c) Creatinine excretion is approximately constant and usually is measured by the Jaffe reaction. A reagent strip (Multistix PRO,™ Bayer, Elkhart, IN, USA) has been developed to detect urine protein, urine creatinine, and urine-protein-to-urine-creatinine ratio (UP/UC ratio). Urine protein and manually calculated UP/UC ratio from the reagent strip results have shown good correlation with quantitative methods in dogs but poor correlation in cats. Widespread use of these reagent strips in clinical practice has not yet occurred.

(d) A reagent strip (CLINITEK® Microalbumin, Bayer, Elkhart, IN, USA) also has been developed to detect microalbuminuria (small quantities of albumin in the urine) in humans; however, test results did not correlate well with quantitative measurement of albumin in dogs.

3. Interpretation of a positive test result (proteinuria)

a. The reagent strip is only a screening test. The UP/UC ratio gives a more quantitative estimate of proteinuria.

b. False-positive reagent strip test results may occur in highly alkaline urine (pH greater than 8.0); proteinuria should be confirmed by acid precipitation or other quantifying tests (Case 34). Reagent strips are not very useful in detecting proteinuria in ruminants because their urine is usually alkaline. Alkaline pH causes the indicator pad to change color despite the presence or absence of protein. False negative readings do not occur in acid urine because of the low pH of the reagent strip buffer.

Free ebooks ==> www.Ebook777.com

c. An occult blood test and urine sediment examination are necessary to distinguish nonrenal from renal proteinuria and to interpret the UP/UC ratio. In the absence of prerenal proteinuria, hemorrhage, and inflammation, the following are true:

(1) UP/UC ratio below 0.5 is considered normal.

(2) UP/UC ratio of 0.5 to 1.0 is questionable for renal proteinuria.

(3) UP/UC ratio greater than 1.0 indicates renal proteinuria.

d. Causes of urogenital proteinuria

(1) Hemorrhage into the urinary tract (Cases 8, 20, 22, 25, 27)

(a) The urine occult blood test is positive, and greater than 5 erythrocytes per high-powered field of view (HPF) usually are observed in the urine sediment.

(b) High urine protein dipstick values occur due to plasma-derived albumin.

(c) Trauma, inflammation, and neoplasia are common causes of hemorrhage or hematuria.

(2) Inflammation within the urinary tract (Cases 4, 8, 20)

(a) Leukocytes (greater than 5 per HPF) are observed in the urine sediment.

(b) Precise location of the inflammation is difficult to discern by examination of urine alone. However, the presence of cellular casts composed of leukocytes suggests that tubular involvement is present.

(c) Bacteria or other pathogens may be present.

(d) Plasma-derived protein concentration seldom exceeds a 2+ reaction, unless the inflammation also is accompanied by hemorrhage.

(3) Renal disease (Cases 13, 18, 19)

(a) Absence of occult blood and significant cellular sediment are typical findings.

(b) Casts may or may not be present.

(c) Primary glomerular disease may cause intense proteinuria (Case 19)

i) Regent strip reactions usually are 3 to 4+.

ii) UP/UC ratio is often greater than 3 and usually greater than 5.

iii) The major protein component is albumin.

iv) Primary diseases in the dog are amyloidosis and glomerulonephritis. Dogs with amyloidosis usually have higher UP/UC ratios (greater than 18) than with glomerulonephritis (5 to 15).

(d) Primary tubular disease causes mild to moderate proteinuria.

i) Reagent strip reactions are less than 2+.

ii) UP/UC ratio is less than 3.

iii) The major protein components are low-molecular-weight globulins.

(e) Proteinuria of renal origin usually is associated with both glomerular and tubular disease.

(f) Absence of proteinuria does not exclude significant renal disease, especially nonglomerular disease.

(4) Prerenal proteinuria

(a) Certain extrarenal factors may cause a transitory, mild proteinuria via increased glomerular permeability (e.g., fever, cardiac disease, central nervous system disease, shock, muscular exertion).

(b) Proteinuria, derived from colostral proteins, occurs in foals, calves, kids, and lambs under 40 hours of age.

(c) High concentrations of low-molecular-weight proteins in the blood may pass through the glomerulus and overwhelm the resorptive capacity of the tubules, causing an overflow proteinuria (e.g., Bence-Jones protein, hemoglobin dimers, myoglobin) (Cases 3, 17).

(5) Trace amounts of protein usually are present in avian urine.

B. Glucose

1. Physiology

a. Glucose is freely filtered by the glomerulus.

www.Ebook777.com

b. Glucose is completely resorbed in the proximal renal tubules, provided that the cells' transport maximum is not exceeded. If blood glucose values exceed the transport maximum, glucosuria will occur. In the following animals, glucosuria can be expected when serum glucose concentrations exceed these levels:

(1) Above 100 mg/dL in the cow
(2) Above 180 mg/dL in the dog
(3) Above 280 mg/dL in the cat
(4) Above 600 mg/dL in birds

2. Methods of measurement

a. Reagent strips employ the glucose oxidase method, which is specific for glucose. Positive values occur when urine glucose is above 100 mg/dL. Reagent strips provide more sensitive detection of glucose than reduction tests, but may be inhibited by ascorbic acid, formalin, and low urine temperature (refrigerated urine specimens). False-positive reagent strip test values may occur in the presence of oxidizing agents such as hydrogen peroxide or chlorine bleach.

b. The copper reduction tablet method semiquantitates glucose, but it also reacts with other reducing substances (e.g., lactose, galactose, pentose, ascorbic acid, conjugated gluconates, salicylates), resulting in false-positive test results. Exposure of these tablets to moisture can lead to a false-negative test result.

3. Interpretation of a positive test result (glucosuria)

a. Hyperglycemia (Chapter 6, Table 6.2) of sufficient magnitude to exceed the tubular transport capacity is the most common cause of glucosuria (Cases 14, 15, 22, 23). Transient hyperglycemia is relatively common in cats and birds with excitement (catecholamine effect) or severe stress (endogenous corticosteroid release).

b. Normoglycemia (with decreased tubular reabsorption of glucose) is a less common cause of glucosuria:

(1) Canine Fanconi-like syndrome (Basenji, Labrador Retriever) and primary renal glucosuria are associated with defective renal tubular absorption.

(2) High dosages of gentamycin or amoxicillin may cause renal tubular damage with glucosuria.

(3) Other types of tubular disease rarely have glucosuria. Thus, the presence of glucosuria is not a reliable measurement of tubular disease.

(4) Collection of blood and urine from a cat or bird that was previously excited or stressed may reveal normoglycemia with glucosuria. Blood glucose concentration can return to the reference interval, but glucose will persist in urine that is stored in the bladder of cats or the cloaca of birds.

C. Ketones

1. Physiology

a. Ketones are freely filtered by the glomerulus.

b. Under normal circumstances, ketones are completely resorbed by the proximal tubules.

2. Methods of measurement

a. Reagent strip and tablet methods use the nitroprusside reaction.

b. The above methods detect acetone and acetoacetic acid, but do not detect β-hydroxybutyrate (the major intermediate in ketosis).

c. False-negative reactions occur when the urine specimen is not fresh or with prolonged exposure of the reagent pad to moisture.

3. Interpretation of a positive test result (ketonuria) (Cases 15, 34)

a. Ketonuria precedes detectable ketonemia (Chapter 6).

(1) Following glomerular filtration, ketones are incompletely reabsorbed by the renal tubular epithelial cells, resulting in ketonuria.

Free ebooks ==> www.Ebook777.com

(2) Routine biochemical profiles do not specifically detect ketones. Their presence in serum is suggested indirectly by titration acidosis and an increased anion gap. Lactic acidosis can produce similar laboratory abnormalities (chapters 5 and 6).

b. Ketonuria is not an indicator of renal disease.

c. Ketonuria indicates excessive fat degradation and/or deficiency in carbohydrate metabolism (negative energy balance) and may occur in the following conditions:

(1) Ketosis of cattle
(2) Pregnancy disease of ewes
(3) Diabetes mellitus
(4) Starvation (particularly young animals)
(5) Low-carbohydrate, high-fat diets

D. Bilirubin (Chapter 7)

1. Physiology

a. Conjugated bilirubin can readily pass through the glomerulus and into the filtrate. Unconjugated bilirubin is bound to serum albumin and cannot pass through the glomerulus. The renal threshold for bilirubin appears to be lower in the dog (particularly males) than in other species.

b. Conjugated bilirubin is not reabsorbed by the renal tubules.

c. In the dog, some conjugated bilirubin may be formed by tubular epithelial cells from absorbed hemoglobin.

2. Methods of measurement

a. Reagent strips use the diazotization method. Discoloration of the urine may obscure color changes in the reagent pad and interfere with reading the test result.

b. The tablet method uses a similar chemical procedure but is more sensitive in detecting bilirubin.

c. The diazotization method is more reactive with conjugated bilirubin and rather insensitive to unconjugated bilirubin.

d. Bilirubin may be oxidized to biliverdin or hydrolyzed to free bilirubin upon exposure to light. Excessive delay in urinalysis may result in a false negative test result.

3. Interpretation of a positive test result (bilirubinuria) (Cases 2, 3, 4, 13, 14)

a. Bilirubinuria indicates obstruction to bile flow and regurgitation of conjugated bilirubin into the blood.

b. Bilirubinuria can be detected prior to bilirubinemia because of the low renal threshold.

c. Increased tubular cell formation of bilirubin in hemoglobinuria and increased hepatic conjugation of bilirubin in intravascular hemolysis may produce bilirubinuria.

d. Trace bilirubinuria may be detected in concentrated urine of dogs because of a low renal threshold for conjugated bilirubin.

e. Any degree of bilirubinuria is significant in cats.

f. Biliverdin is the main component of heme catabolism in birds, although some species (e.g., ducks) can produce a minimal quantity of bilirubin. Urine biliverdin does not react with the reagent pad or tablet methods. Therefore, any reactivity for bilirubin in birds is unexpected, but rarely may indicate hepatic or hemolytic disease.

E. Occult blood test

1. Physiology

a. Hemoglobin must exceed the binding capacity of plasma haptoglobin and split into dimers before it can pass through the glomerular filter. Subsequently, the hemoglobin dimers must exceed the absorptive capacity of the tubules before hemoglobinuria occurs.

b. Myoglobin is small enough (MW = 17,800 Da) to readily pass the glomerular filter. Because myoglobin is only one-fourth the size of hemoglobin (MW = 68,800 Da), it is cleared from the plasma rapidly.

www.Ebook777.com

2. Methods of measurement. The commonly used reagent strip and tablet methods are based on the peroxidase-like properties of hemoglobin and myoglobin with the subsequent oxidation of ortho-toluidine to a blue-colored derivative.
3. The occult blood test is much more sensitive for hemoglobin than are the urine protein tests. Tests for occult blood usually react maximally (4+) before tests for protein detect the same amount of hemoglobin.
4. Causes of a positive occult blood test and their differentiation
   a. Hematuria (Cases 8, 20, 22, 25, 27) is differentiated by the following:
      (1) Red, cloudy urine that usually clears with centrifugation
      (2) Erythrocytes in the urine sediment. A reagent in the dipstick pad will lyse intact RBCs for subsequent hemoglobin detection. In addition, some of the erythrocytes in the urine will lyse and release hemoglobin.
      (3) Absence of clinical or laboratory evidence of hemolytic anemia or muscle disease
   b. Hemoglobinuria (Case 3) is differentiated by the following:
      (1) Red to brown urine that does not clear on centrifugation
      (2) Absence of erythrocytes in the urine sediment. Erythrocytes suspended in urine with a very low specific gravity (less than 1.006) or in aged urine may lyse and mask pre-existing hematuria.
      (3) Concomitant red discoloration of the plasma (hemoglobinemia). Free hemoglobin will discolor plasma before it saturates serum haptoglobin or causes hemoglobinuria.
      (4) Evidence of anemia, particularly an intravascular hemolytic type
      (5) Absence of clinical or laboratory evidence of muscle disease
      (6) Addition of saturated ammonium sulfate solution will remove the color by precipitation of hemoglobin. Ammonium sulfate solution will not precipitate myoglobin and the urine remains discolored. Spectrophotometric analysis is a better technique to differentiate hemoglobinuria from myoglobinuria.
   c. Myoglobinuria (Case 17) is characterized by the following:
      (1) Red to brown urine that does not clear on centrifugation
      (2) Absence of erythrocytes in the urine sediment
      (3) Clear, normally colored plasma. Myoglobin does not bind significantly to serum proteins and is excreted in the urine before reaching concentrations that discolor plasma.
      (4) Absence of clinical or laboratory evidence of an anemia
      (5) Clinical or laboratory evidence of muscle disease (Chapter 10)
   d. Oxidizing agents in urine may give a false positive test result for blood.

F. Urobilinogen (Chapter 7)
1. Physiology
   a. Urobilinogen is formed in the intestine by bacterial action on conjugated bilirubin.
   b. Urobilinogen is absorbed from the intestine into portal blood, where it is recycled through the liver and excreted in bile. A small amount of urobilinogen passes through the glomerular filter and into the urine.
2. Methods of measurement
   a. Urobilinogen generates a cherry-red color in Ehrlich's reagent (Ehrlich's diazo reaction is the formation of a red color from the action of diazo-benzenesulfonic acid and ammonia upon certain aromatic substances, such as urobilinogen, that are found in the urine). This analytical method is semiquantitative.
3. Interpretation of findings
   a. The presence of urobilinogen in urine indicates a patent bile duct.
   b. Absence of urobilinogen may indicate complete bile duct obstruction. However, because of the instability of urobilinogen and diurnal variation in the excretion of this substance, many normal animals lack detectable urobilinogen in their urine. In aged urine, urobilinogen is rapidly oxidized to urobilin, resulting in a false negative test result.

Free ebooks ==> www.Ebook777.com

c. Increased concentrations of urobilinogen may occur in hemolytic diseases and with reduced functional hepatic mass. In the latter condition, bile flow persists, but lower concentrations of absorbed urobilinogen are removed from the portal blood by the hepatocytes.
d. The correlation between urine urobilinogen concentration and hepatobiliary disease is poor in animals.

G. Hydrogen ion concentration (pH)
1. Methods of measurement
a. A variety of reagent strips impregnated with chemical indicators are available for the determination of pH. Determination of urine pH with a pH meter is more accurate, but is expensive and less practical in a clinical setting.
b. A fresh sample is necessary; urine becomes alkaline on standing because CO_2 is lost and bacteria convert urea into ammonia.
2. Interpretation
a. Urine pH is the result of renal regulation of blood bicarbonate and H^+ concentrations; however, urine pH should not be used alone to evaluate acid-base status.
b. Dietary effects on urine pH in mammals
(1) An acidic pH (below 7.0) occurs with high-protein, meat-based diets (meat and milk in carnivores and nursing animals) and complete anorexia in both carnivores and herbivores.
(2) An alkaline pH (above 7.0) occurs with vegetable diets in herbivores and may occur with vegetable-based diets in carnivores.
c. Urinary tract infections with certain types of bacteria yield a higher pH than expected because bacteria degrade urea to ammonia (Case 34).
d. Urine pH determines the types of casts, crystals, and uroliths that may form in urine.
e. Therapy may affect urine pH, and urine pH subsequently may affect drug efficiency in combating urinary tract infection.
f. The pH of avian urine ranges from 6.0 to 8.0. Diet affects the pH of avian urine as it does in other animals.
g. Renal tubular acidosis (RTA)
(1) RTA encompasses disorders that affect the kidneys' ability to do the following:
(a) Secrete hydrogen ions (Type I, distal RTA)
(b) Retain bicarbonate ions (Type II, proximal RTA)
(c) Secrete H^+ and K^+ in the collecting ducts (Type IV RTA) secondary to aldosterone deficiency or resistance
(2) In type I RTA, urine pH is above 6.0.
(3) In type II and type IV RTA, urine pH is below 6.0.
h. Metabolic alkalosis with aciduria (paradoxical aciduria) is discussed in Chapter 5.
i. Improper technique with reagent strips may allow the acid buffer from the protein pad to flow into the pH pad. A false low pH test result will occur.

H. Nitrite
1. This test has been used to screen for certain bacteria.
2. The reaction is based on the principal that nitrate (normally found in urine) is reduced to nitrite by nitrate-reducing bacteria.
3. This reagent strip test for use in humans has not proven to be reliable with animal urine specimens because false negative test results often occur.

I. Leukocyte esterase activity
1. This test has been used in humans to detect leukocytes in the urine, indicating that inflammation or infection is present.
2. This reagent strip test is unreliable with urine from animals; sediment examination is required to detect pyuria.

www.Ebook777.com

J. Enzymes

1. Enzymatic activity in urine may detect renal tubular damage, provided that the following are true:

a. Similar plasma enzymes are large enough to be restricted from the glomerular filtrate. Therefore, these enzymes are not normally present in the urine.

b. The enzyme in question has a high activity within or on the surface of renal tubular epithelial cells.

c. Enzymatic activity can be measured reliably in the urine.

2. Twenty-four-hour urine activity and enzyme activity: urine creatinine ratios of gamma glutamyltransferase (GGT), N-acetyl-beta-D-glucosaminidase (NAG), alkaline phosphatase, and β-glucuronidase have been used to detect renal tubular damage. Although Rules Based Medicine (Austin, TX, USA) has developed a new research biomarker panel for early detection of renal toxicity in humans and rats, these biomarkers have not yet been tested in domestic animals or used in a clinical setting.

K. Canine bladder tumor antigen test (V-BTA™, Alidex, Inc., Redmond, WA, 98052 USA)

1. The V-BTA™ assay is a latex agglutination screening test to detect transitional cell carcinoma antigens in canine urine.

2. False-positive test results may be observed in urine samples with 4+ proteinuria, 4+ glucosuria, and greater than 30 to 40 RBC and/or WBC/HPF.

V. SEDIMENT EXAMINATION (FIGURE 9.1)

A. Principles

1. Negative or normal reagent strip test results do not always mean that the urine sediment will be normal. A complete urinalysis should always include microscopic examination of the sediment.

2. Proper technique is necessary for accurate sediment evaluation.

a. Low centrifuge speeds are necessary to prevent destruction of some constituents, especially casts.

b. If necessary, the urine supernatant may be re-evaluated with the urine dipstick or with semiquantitative methods after centrifugation.

c. The supernatant is decanted and the sediment is resuspended in the remaining urine by gentle, thorough mixing. The volume of urine used for sediment resuspension should be consistent. The quantity of sediment is related to the volume and concentration of urine being centrifuged. Therefore, a standard volume of urine should be centrifuged for sediment evaluation. Abnormal findings should be evaluated with regard to urine specific gravity.

d. A drop of resuspended urine sediment is placed on a glass slide and coverslipped for microscopic examination.

3. To examine unstained sediment microscopically, maximum contrast is needed and may be obtained by dimming the microscope light, closing the iris diaphragm, and/or lowering the condenser. Stains are available for use with urine sediment that may enhance contrast for detection and identification of inflammatory cells, bacteria, and neoplastic cells. The urine sediment is examined at both low- (10×) and high- (40 to 45×) powered fields (HPF) of view.

4. The method used to collect the urine specimen influences the type and amount of sediment present. Voided (free catch) specimens may be more cellular, have bacterial contamination, and have discharges from the reproductive tract. Catheterized specimens may have increased transitional cell content and iatrogenic hemorrhage. Cystocentesis specimens have the least extraneous contamination and are more specific for changes in the urinary tract.

B. Epithelial cells (Figure 9.1)

1. Epithelial cells can originate from the kidneys, ureters, bladder, urethra, and reproductive tract.

Free ebooks ==> www.Ebook777.com

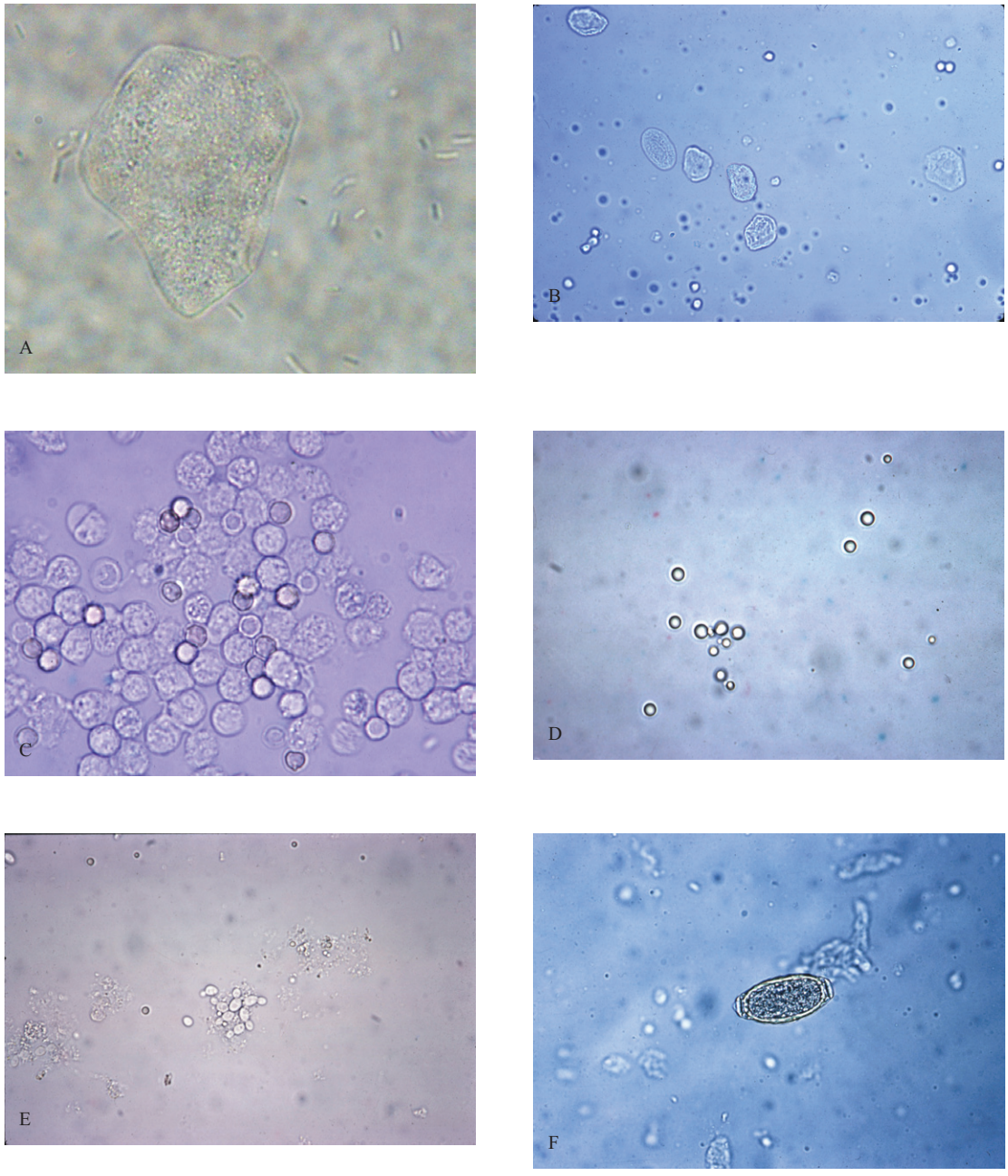
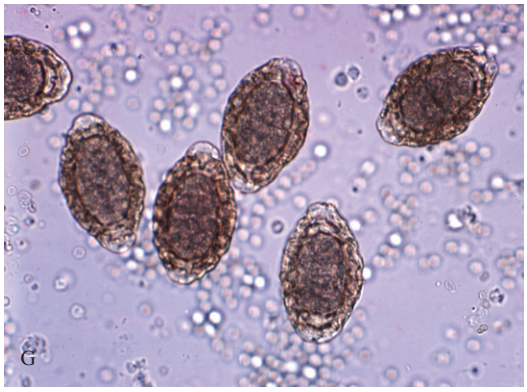
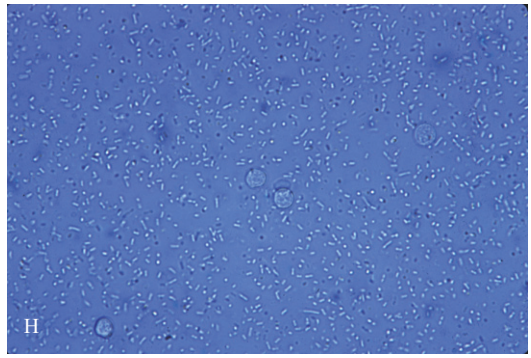
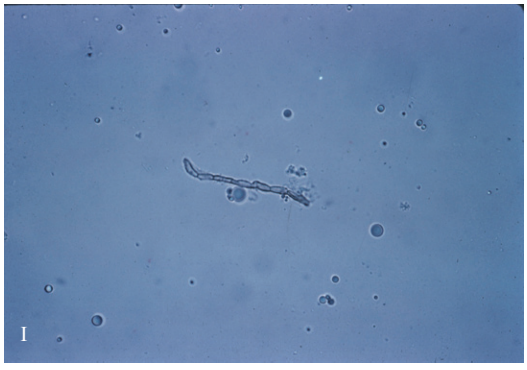


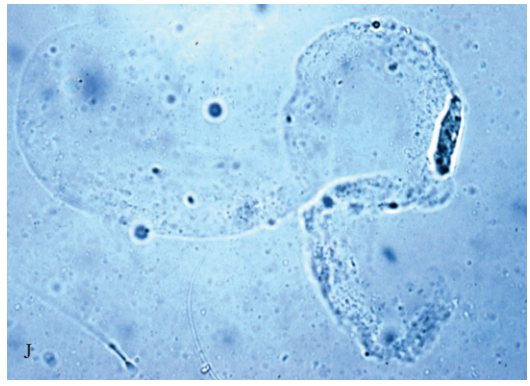
FIGURE 9.1. Urinary sediment. A. squamous epithelial cells, B. transitional epithelial cells, C. erythrocytes and leukocytes, D. fat droplets, E. *Candida* sp. yeasts, F. *Capillaria plica* ovum; continued.

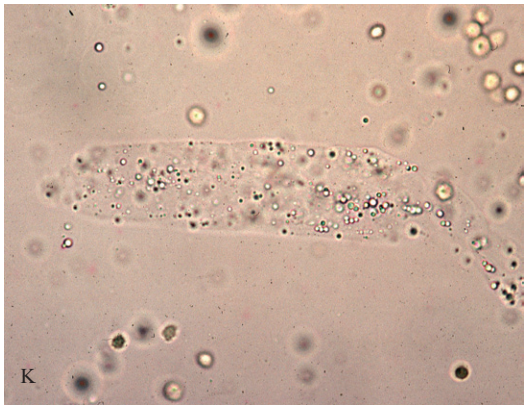
www.Ebook777.com











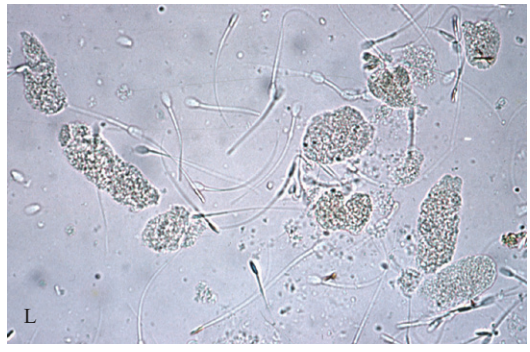
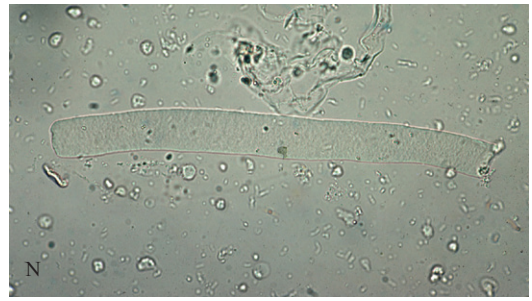
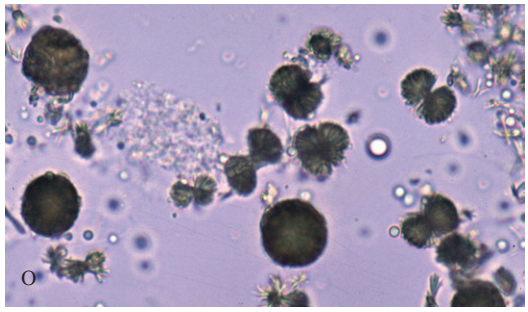


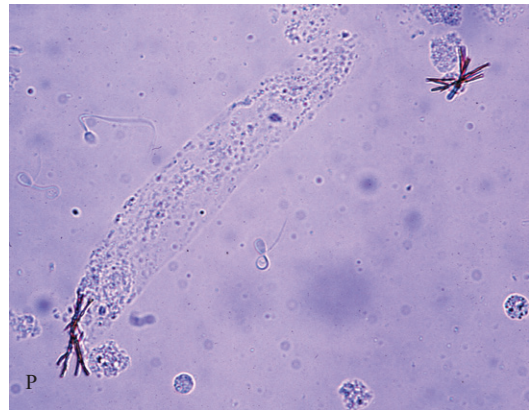
FIGURE 9.1. continued. G. *Dioctophyma renale* ova, H. bacteria and leukocytes, I. fungal hypha, J. mucus thread, K. hyaline cast, L. granular cast and spermatozoa; continued.

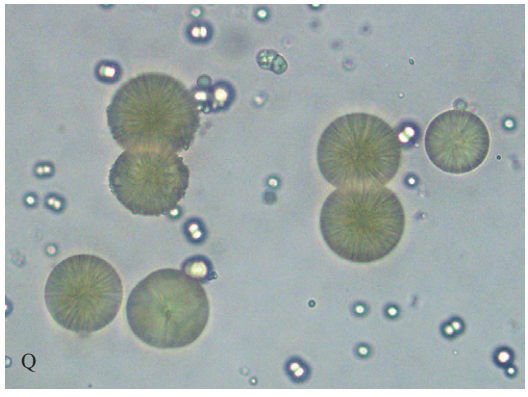
Free ebooks ==> www.Ebook777.com











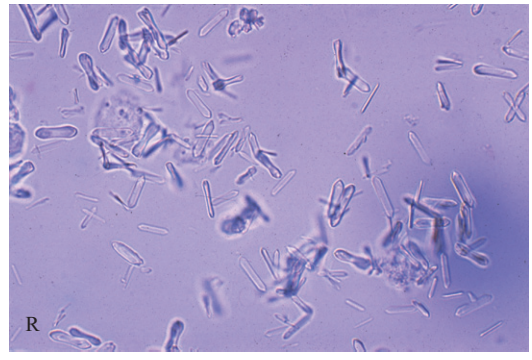
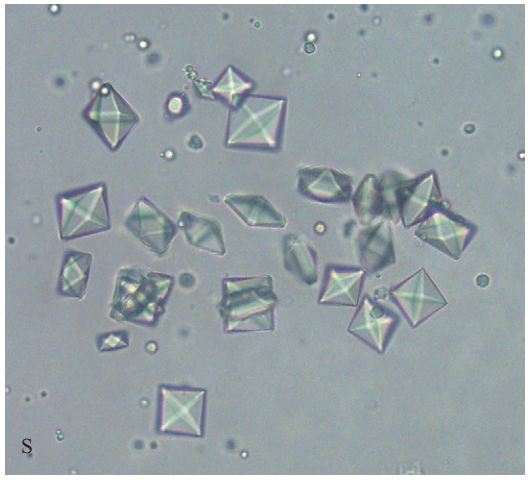
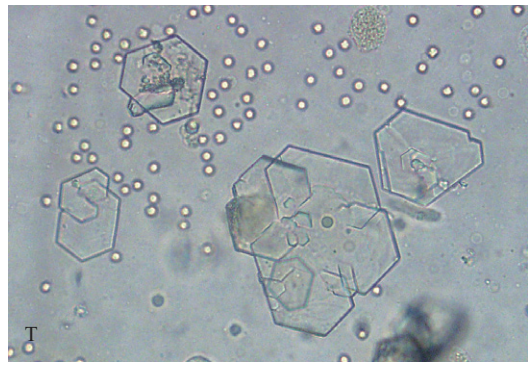
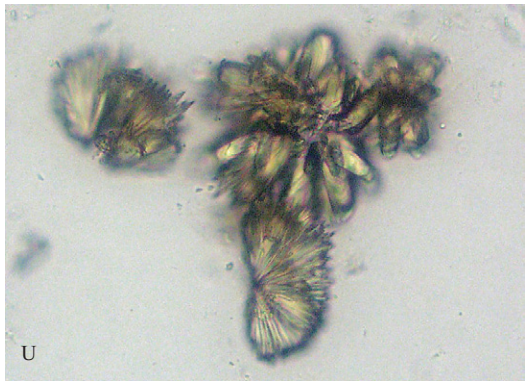


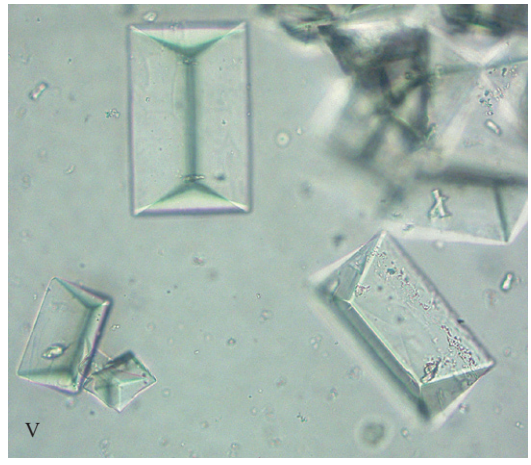
FIGURE 9.1. continued. M. cellular cast with hemoglobin staining, N. waxy cast, O. ammonium biurate, P. bilirubin with hyaline cast, spermatozoa, and leukocytes, Q. calcium carbonate, R. calcium oxalate monohydrate (ethylene glycol toxicosis); continued.

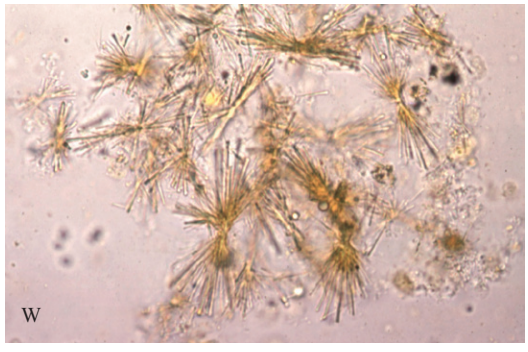
www.Ebook777.com











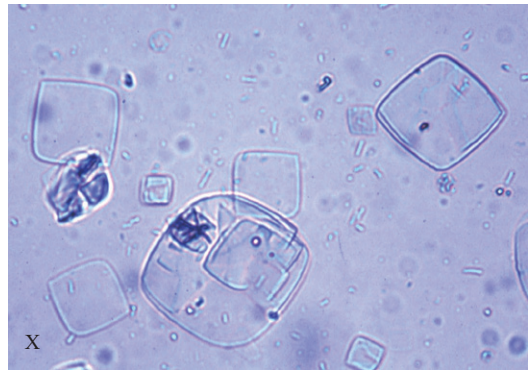
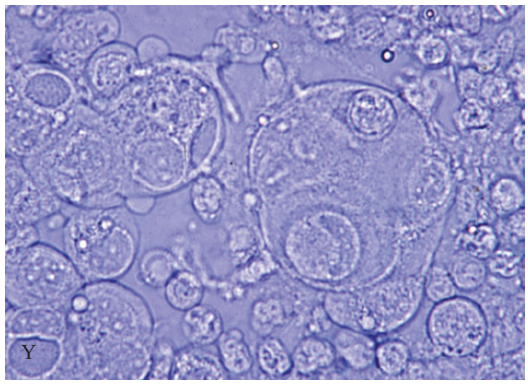


FIGURE 9.1. continued. S. calcium oxalate dihydrate, T. cystine crystals, U. sulfonamide crystals, V. triple phosphate, W. tyrosine, X. uric acid; continued.

Free ebooks ==> www.Ebook777.com



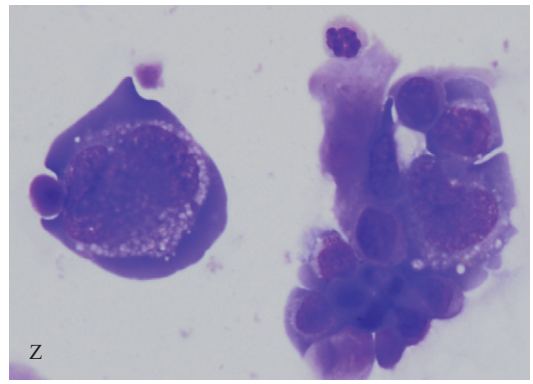


FIGURE 9.1. continued. Y. transitional cell carcinoma (wet mount), Z. transitional cell carcinoma (Wright's stain).

2. Squamous epithelial cells are large with irregular, angular margins and small nuclei. Bacteria may be observed on their surfaces. They desquamate from the urethra and vagina or prepuce and indicate contamination.

3. Transitional epithelial cells are variably sized, oval, spindle, or caudate (tailed). They originate from the proximal urethra, urinary bladder, ureter, and renal pelvis. They may occur in groups, especially in catheterized samples, but are of little diagnostic importance unless neoplastic (e.g., transitional cell carcinoma).

4. Renal epithelial cells are small, round, and slightly larger than leukocytes. They are derived from the renal tubules. Renal epithelial cells usually are degenerative; they may be difficult to identify and differentiate from leukocytes.

C. Erythrocytes (Cases 8, 20, 22, 25, 27, 34) (Figure 9.1)

1. Erythrocytes are round to biconcave, slightly refractile, lack internal structure, and may have a light green tint in unstained wet mounts.

2. More than 5 RBCs/HPF indicates hemorrhage (hematuria), which may be traumatic or inflammatory in nature (see Occult Blood Test earlier in this chapter).

3. Erythrocytes often appear shrunken or crenated in concentrated urine (high specific gravity).

4. Erythrocytes may lyse or become ballooned ghost cells in dilute urine (specific gravity less than 1.006). They also will deteriorate in aged and alkaline urine.

5. Structures that may be mistaken for erythrocytes include fat droplets and yeasts.

a. Fat droplets are variably sized, highly refractile, less dense, and float immediately beneath the coverslip (out of the plane of focus of the cells).

b. Yeasts are oval, often exhibit budding, and are more variable in size than RBCs.

D. Leukocytes (Cases 4, 8, 20, 34) (Figure 9.1)

1. Leukocytes, usually neutrophils, appear round and granular. They are larger than erythrocytes and smaller than epithelial cells. Leukocytes decompose in old urine and may lyse in hypotonic or alkaline urine.

2. More than 5 leukocytes/HPF indicates urogenital tract inflammation (pyuria) that may or may not be septic.

3. Leukocytes frequently are associated with bacteriuria, but significant bacteriuria may occur without pyuria. Intracellular bacteria are difficult to detect without examining a stained sediment preparation.

4. Glitter cells are leukocytes that have highly refractile intracytoplasmic granules that appear to shimmer.

www.Ebook777.com

E. Casts (cylinduria) (Cases 17, 34) (Figure 9.1)

1. General features of casts

a. Casts are elongated structures comprised of a matrix of Tamm-Horsfall protein, a high-molecular-weight mucoprotein that is produced by the distal tubular epithelial cells.

b. Casts are formed in distal tubules where urine is more acidic; they may dissolve in alkaline urine.

c. Structures present in the tubule at the time of cast formation may be embedded in the cast.

d. Although casts indicate some tubular change, they do not correlate with the severity of change. Casts can be observed in the urine of apparently healthy individuals.

e. Casts tend to be discharged into urine intermittently. They may or may not be observed in a single urinalysis.

f. The absence of casts does not exclude renal disease.

g. Casts are classified according to their major component.

2. Types of casts

a. Hyaline casts are colorless, homogenous, semi-transparent, and difficult to detect, even with reduced light. They are comprised mostly of Tamm-Horsfall protein.

b. Granular casts are the most common type of cast observed. They are comprised of mucoprotein, plasma proteins, degenerated cells, and, occasionally, tubular debris.

c. Cellular casts

(1) Epithelial cell casts contain cells that have desquamated from the renal tubules.

(2) WBC casts occur in renal inflammation.

(3) RBC casts occur in renal hemorrhage and inflammation.

d. Waxy casts are wide structures without granular contents that usually have sharply broken or square ends and notched edges. They evolve from degenerating cellular and granular casts. Waxy casts are formed in the large collecting tubules and indicate chronic tubular lesions, severe urine stasis, and a poor clinical prognosis.

e. Fatty casts contain fat globules derived from degenerating tubular epithelial cells. They are observed most commonly in cats.

f. Pseudocasts are aggregates of amorphous crystals, debris (fiber, glass particles), bacteria, or other structures that are not embedded in a protein matrix. They mimic true casts.

F. Mucus (Case 17) (Figure 9.1)

1. Mucus appears as narrow, twisted, ribbon-like, homogenous threads and indicates urethral irritation or genital secretions.

2. Mucus is a normal finding in equine urine.

G. Fat (Figure 9.1) is recognized as variably-sized, highly refractile droplets. Because they are less dense than water, fat droplets float underneath the coverslip of urine sediment preparations and are in a different focal plane than cells and casts.

1. Fat droplets may be stained directly with Sudan III. Because new methylene blue stain is a water-based dye solution, fat appears as highly refractile, unstained droplets in these preparations.

2. Fat droplets usually are of no pathologic significance in urine specimens. These droplets are common in feline urine due to the high lipid content of renal tubular epithelial cells.

H. Bacteria (Cases 20, 34) (Figure 9.1)

1. Identifying features

a. Bacilli (rods) may occur singly or in chains. They are readily identified in unstained wet mounts of urine sediment and are usually accompanied by leukocytes.

b. Cocci (round bacteria) are more difficult to identify in unstained preparations, unless present in chains, because they resemble small crystals or debris.

c. Brownian motion of small particles should not be misidentified as cocci or motile bacteria.

Free ebooks ==> www.Ebook777.com

d. Romanowsky- or Gram-stained preparations of urine sediment are superior to wet mounts for detecting bacteria, especially cocci. Stained preparations should be used to confirm questionable bacteriuria.

2. Significance of bacteriuria

a. Greater than 30,000 bacterial rods/mL usually must be present before microscopic detection of bacterial cells is possible in wet mounts of urine sediment. In contrast, even greater numbers of cocci must be present for microscopic detection of these organisms.

b. Urine is normally sterile only from the kidney to the mid-urethra; therefore, bacteria observed in voided samples may represent contamination by normal flora of the lower urogenital tract. Contamination also may occur in catheterized samples, particularly those obtained from females.

c. Quantitative and qualitative urine cultures are necessary to detect clinically significant bacteriuria. Obviously, the collection vessel must be sterile if microbiological culture is to be performed.

d. In most instances, bacteriuria in birds cannot be distinguished from fecal contamination.

I. Sperm may be observed in voided urine from males and from recently inseminated females.

J. Parasite ova (*Stephanurus dentatus*, *Dioctophyma renale*, and *Capillaria plica*) and microfilaria (*Dirofilaria immitis*) are observed infrequently in urine specimens (Figure 9.1).

K. Fungi. Segmented hyphae or budding yeasts may be found. Yeasts usually are contaminants in stale urine specimens. Fungi have rarely been observed with disseminated *Aspergillus terreus* infection in German Shepherd dogs.

L. Algae. *Prototheca* sp. has been observed in urine sediment of dogs with disseminated algal infection. These organisms are variably sized with a thick cell wall and internal compartmentalization resulting from binary fission.

M. Crystalluria (Figure 9.1)

1. Crystalline precipitation of solutes depends upon pH, temperature of the urine, and degree of solubility and concentration of the crystalloid.

2. Crystalluria indicates saturation of urine with a crystalloid material but is not always indicative of urolithiasis.

3. Crystals are identified by their shape, color, and solubility in acid or alkaline solutions.

4. Crystals found in uroliths include, but are not limited to, magnesium ammonium phosphate (triple phosphate, struvite), calcium oxalate, calcium phosphate, urate, cystine, silica, and calcium carbonate.

5. Crystals found in urine sediment include the following:

a. Ammonium biurate (ammonium urate), which can occur with portosystemic venous shunts or other hepatic diseases where hyperammonemia exists (Case 13). Ammonium biurate crystals are yellow to brown spheres with irregular pointed projections ("thornapple" crystals). These crystals occasionally lack the characteristic projections (smooth form).

b. Amorphous urates or phosphates are ill-defined aggregates of crystals that may form pseudocasts. Urates are common in birds.

c. Bilirubin crystals are yellow to amber and resemble a stack of twigs or "antler" configurations. They are common in canine urine but abnormal in the urine of other species.

d. Calcium carbonate crystals are dumbbell-shaped or spherical with radiating spokes. They are considered a normal finding in equine urine.

e. Calcium oxalates

(1) Calcium oxalate monohydrate crystals are common in ethylene glycol toxicosis (Case 18) or other oxalate poisonings. These crystals are spindle shaped ("picket fence" or "hempseed" appearance).

www.Ebook777.com

(2) Calcium oxalate dihydrate crystals are found in urine of healthy individuals and occasionally in ethylene glycol toxicosis. They are octahedrons or envelope-shaped crystals, but may occasionally form cubes.

(3) Ethylene glycol toxicosis may occur without oxalate crystalluria.

(a) A colorimetric commercial kit (Ethylene Glycol Test Kit, Allelic Biosystems, Kearneysville, WV 25430) is available to detect ethylene glycol in blood within 12 hours of ingestion before crystalluria is evident.

(b) A positive test result is the development of a reddish-violet color that is more intense than the control specimen (greater than 50 µg/mL).

f. Cholesterol crystals are associated with cell membrane degradation and with some renal diseases. The crystals are parallelograms with a notched corner.

g. Cystine crystals are hexagonal and usually result from altered protein metabolism (e.g., congenital cystinuria of dogs).

h. Drugs

(1) Sulfonamide crystals are drug induced. These crystals may form in acid urine of animals that are dehydrated or have restricted access to water. Sulfonamide crystals may vary in morphology. They usually are spherical with radiating spokes and sometimes ragged margins. These crystals appear darker and slightly brown when compared to calcium carbonate crystals. The lignin test can confirm the presence of sulfonamides. This test is performed by placing a drop of urine sediment on newspaper and adding a drop or two of 25% hydrochloric acid. If sulfa drugs are present, a bright yellow-orange color is produced.

(2) Ampicillin crystals form long, needle-like arrays.

i. Triple phosphate (magnesium ammonium phosphate, struvite) crystals are three- to six-sided "coffin lids." They occasionally exhibit a "fern leaf" morphology, especially if they are dissolving.

j. Tyrosine crystals, which may be associated with liver disease in the dog, are colorless to yellow needles arranged like sheaves of wheat.

k. Uric acid crystals are usually parallelograms with rounded corners, but can be pleomorphic in shape, forming "lemon drops," rhomboids, or rosettes. These crystals are observed commonly in urine from Dalmatian dogs. They are considered a normal finding in this animal.

RENAL ABNORMALITIES DETECTED BY LABORATORY TESTS

I. DEFINITIONS

A. Renal disease

1. Renal disease is defined as the occurrence of morphologic renal lesions of any size or severity or any biochemical abnormalities related to renal function.

2. Because of the extensive reserve capacity of the kidney, significant renal disease may be present in the absence of clinical signs or laboratory abnormalities.

3. In some instances, signs of renal disease, such as proteinuria and casts, may not be accompanied by clinical evidence of loss of renal function.

4. The various parts of the nephron are so interrelated that disease in the glomerulus often results in tubular disease and vice versa.

B. Renal failure

1. Renal failure is present when clinical signs or laboratory abnormalities are observed that are caused by reduced renal function.

2. Failure of renal function occurs only after substantial loss of nephrons.

a. Quantitation of renal function is based on the intact nephron hypothesis, which states that decline in renal function is the result of a decreased number of functioning nephrons rather than decreased function of individual nephrons.

Free ebooks ==> www.Ebook777.com

b. Renal functions that can be measured include the following:
(1) Excretion of nitrogenous wastes (e.g., BUN, creatinine)
(2) Acid-base balance (TCO_2, blood gases)
(3) Regulation of body water and solute content of urine (e.g., urine specific gravity, osmolality)
(4) Degradation of certain compounds (e.g., plasma amylase and lipase)
(5) Erythropoietin secretion
c. Only one function may be lost; however, multiple functions usually are lost concurrently.

C. Azotemia
1. Azotemia is an excess of urea or other non-protein nitrogenous compounds in the blood.
2. Causes of azotemia may be pre-renal, renal, or postrenal (see Blood Urea Nitrogen later in this chapter).

D. Uremia
1. Uremia is a complex of clinical signs observed in renal failure. Uremia also may be observed in pre-and post-renal azotemia. If clinical signs of disease are absent, an animal with azotemia is not uremic.
2. Clinical signs associated with uremia include anorexia, vomiting, diarrhea, gastrointestinal hemorrhage, ulcerative stomatitis, weakness, lethargy, muscle tremors, convulsions, and terminal coma. Bruxism occurs in cattle.

II. EVALUATION OF RENAL FUNCTION

A. Urine concentration tests
1. Rationale
a. Dehydration increases plasma osmolality, which stimulates the release of antidiuretic hormone (ADH) by the pituitary gland. ADH acts on the collecting tubular epithelial cells, causing resorption of water and concentration of the urine (increased urine specific gravity).
b. Clinical indications for using the urine concentration test include the following:
(1) Polydipsia and polyuria in animals lacking azotemia, clinical evidence of dehydration, and biochemical evidence of disease.
(2) Repeated random urine samples from a nonazotemic animal in which the urine persistently has a low or isosthenuric specific gravity (1.008 to 1.030 in the dog, 1.008 to 1.035 in the cat, and 1.008 to 1.025 in the horse and cow).
c. The urine concentration test is contraindicated in the following conditions:
(1) Azotemia or uremia. A diagnosis of renal disease is already established if azotemia accompanies dilute urine (postrenal azotemia is still a diagnostic consideration). Prerenal azotemia is associated with concentrated urine.
(2) Dehydration. Maximal stimulation of ADH release is already in effect; further water deprivation is unnecessary.
(3) Severe debilitation.
(4) Evidence of other metabolic diseases that can cause polyuria and polydipsia. (Table 9.1)
2. Types of concentration tests
a. Abrupt water deprivation test
(1) The animal is weighed, abruptly deprived of water, and urine specific gravity is monitored. The test is stopped if adequate concentrating ability (i.e., specific gravity greater than 1.030 in the dog, greater than 1.035 in the cat, greater than 1.020 in bird, and greater than 1.025 in the cow and horse) is demonstrated. The test also is terminated if undesirable clinical signs develop or if 5% of body weight is lost (which indicates that adequate stimulus for ADH release and subsequent urine concentration has occurred).

www.Ebook777.com

(2) In cattle, three to four days of water restriction are required for maximum concentration of urine because of the large reservoir of water in the rumen.

b. Gradual water deprivation test

(1) This test is suggested to be of value when polyuria is associated with medullary washout of solute. In this situation, medullary hypertonicity must be re-established before the renal tubules can respond to ADH and the abrupt water deprivation test.

(2) While monitoring body weight, the animal is progressively deprived of water until it is completely withheld; the guidelines of the abrupt water deprivation test are then followed.

c. ADH concentration test

(1) This test may be used when water deprivation poses a risk to the patient. This concentration test has been used primarily in the dog and evaluates renal response to exogenous ADH. The ADH concentration test also may be used after the water deprivation test to diagnose pituitary-associated diabetes insipidus.

(2) An exogenous source of ADH is given to stimulate water reabsorption and urine concentration.

(3) Renal concentrating ability appears to be more effective after water deprivation than after injection of ADH.

3. Interpretation of concentration test results

a. A urine specific gravity greater than 1.030 in the dog, greater than 1.035 in the cat, greater than 1.020 in the bird, and greater than 1.025 in the horse and cow indicates adequate concentration of urine.

b. Causes of an abnormal concentration test include the following:

(1) Renal disease (Cases 3, 10, 15, 17, 18, 19, 24)

(a) Approximately two-thirds of the nephrons are nonfunctional before abnormal concentrating ability of the kidney can be demonstrated.

(b) Impaired concentrating ability usually precedes increases in blood urea nitrogen (BUN) or serum creatinine concentration.

(c) In the cat and in the early stages of primary glomerular disease in any species, azotemia may occur simultaneously with or precede concentration abnormalities.

(2) Pituitary diabetes insipidus

(a) Pituitary disease causes a lack of ADH secretion. The renal tubules are normal but are not stimulated to reabsorb water.

(b) The specific gravity is usually in the 1.001 to 1.007 range because the kidney can still reabsorb solute.

(c) Affected animals respond to exogenous ADH by concentrating the urine.

(3) Nephrogenic diabetes insipidus

(a) The renal tubules are refractory to ADH stimulation via water deprivation or exogenous administration of ADH. This may be a primary tubular defect or a secondary effect of certain biochemical abnormalities (e.g., hyperadrenocorticism, hypercalcemia, endotoxemia) (Case 8).

(b) Other renal function tests are normal.

(4) Diseases that cause polyuria and medullary washout

(a) There is no response to the abrupt water deprivation test because the medulla is hypotonic from the loss of solute.

(b) The gradual water deprivation test is indicated. Urine concentration will occur after medullary hypertonicity is re-established.

B. Blood urea nitrogen (BUN)

1. Basic concepts of metabolism

a. Small quantities of urea are ingested and absorbed from the large intestine.

b. The majority of urea in plasma is synthesized by the liver. Specifically, the hepatic urea cycle synthesizes urea from ammonia that is a waste product of protein catabolism.

Free ebooks ==> www.Ebook777.com

c. Urea is not found in feces because it is either absorbed or converted to ammonia by urease-containing bacteria.

d. Once urea enters the vascular system, it passively diffuses throughout the total body water compartment. Approximately 90 minutes are required for equilibrium to be established.

e. BUN and serum urea nitrogen are the same concentration because of equilibration within the total body water compartment. Erythrocytes, plasma, and serum have the same concentration of urea.

2. Urea excretion

a. The kidney is the most important route of urea excretion.

(1) Urea concentration in the glomerular filtrate is the same as that of the blood. Filtration of urea is a simple process that does not require expenditure of energy. Increased BUN concentration is the result of diminished glomerular filtration.

(2) Urea passively diffuses with water from the tubular lumen back into the blood.

(a) The amount of urea absorbed is inversely related to the rate of urine flow through the tubules.

(b) At the highest urine flow rate, approximately 40% of the filtered urea is reabsorbed. If urine flow is decreased (e.g., dehydration, obstruction), more urea is reabsorbed (up to 70%) and the blood urea concentration increases.

(c) Urea, in the presence of ADH, diffuses from the collecting tubules into the interstitium, where it comprises part of the medullary concentration gradient.

b. Saliva, gastrointestinal tract, and sweat are other routes of urea excretion. Excretion of urea by these routes increases when the BUN concentration increases.

(1) Excretion of urea by the gastrointestinal route is futile in simple-stomached animals because almost all urea is degraded by urease-containing bacteria to ammonia. The ammonia is reabsorbed by the intestine and used to resynthesize urea in the liver.

(2) In ruminants, urea that is excreted into the rumen (or ingested in the diet) is degraded by the microflora to ammonia. The ammonia subsequently is then used to synthesize amino acids for protein production.

(3) Urea excretion in ruminants is governed by nitrogen intake. Animals that are on a nitrogen-deficient diet or that have severe anorexia excrete almost all blood urea via the GI tract and very little via the kidneys. Therefore, BUN can be within the reference interval in some ruminants with severe renal disease.

3. Methods of measurement

a. Reagent strips that employ urease may be used to estimate BUN concentration, but this method of analysis is inaccurate.

b. Chromatographic strip tests are based on ammonia release, which produces a color change. These strip tests allow semiquantitation of BUN concentration.

c. Colorimetric methods are preferred and are quantitative.

4. Interpretation of increased BUN concentration

a. Prerenal azotemia (Cases 9, 14, 27)

(1) Increased protein catabolism secondary to small bowel hemorrhage, necrosis, starvation, prolonged exercise, infection, fever, and corticosteroids (endogenous production or exogenous administration) may cause mild increases in BUN via increased hepatic synthesis of urea. Creatinine concentration is not increased.

(2) High-protein diets may cause mild increases in BUN concentration in non-fasting, healthy animals. However, high-protein diets may precipitate a more significant elevation in BUN concentration in animals with occult renal disease. Creatinine concentration generally is not increased unless occult renal disease is present.

(3) Decreased renal perfusion reduces the glomerular filtration rate (GFR) and causes azotemia. This mechanism occurs with shock, dehydration, and cardiovascular disease. The kidney is stimulated to concentrate the urine. Because the tubular flow rate of urine is lower

www.Ebook777.com

and urea reabsorption is greater, marked elevation in BUN concentration may be observed, particularly in cattle. Creatinine concentration also is increased.

(4) In most species, prerenal azotemia occurs more frequently than renal azotemia.

(5) Diseases causing pre- or postrenal azotemia may secondarily affect the kidneys and eventually cause renal azotemia.

(6) Urine specific gravity is high because most situations causing or associated with prerenal azotemia also stimulate ADH secretion. Functional kidneys respond by concentrating the urine. An exception is hypoadrenocorticism.

(7) The urine osmolality to plasma osmolality ratio is high, while urine sodium concentration is low (less than 10 mEq/L).

b. Renal azotemia (Cases 3, 10, 15, 17, 18, 19, 24)

(1) Renal azotemia occurs when approximately three-fourths of the nephrons are nonfunctional. GFR is decreased significantly with insufficient excretion of urea and creatinine.

(a) Therefore, BUN is not a sensitive indicator of renal disease until the renal functional mass has been reduced to the point of azotemia.

(b) Once renal azotemia is present, the BUN concentration approximately doubles each time the remaining functional renal mass is halved. Modest increases in BUN concentration are highly significant at this stage of disease.

(2) Azotemia usually follows concentrating abnormalities in the normal progression of renal failure.

(a) Although the animal is azotemic due to the decreased GFR, and maximal stimulus for urine concentration is present, the kidneys are still unable to concentrate the urine.

i) Cats are an exception to this rule; some concentrating ability of the feline kidney may persist after azotemia has developed.

ii) Animals with primary glomerular disorders may develop azotemia before concentrating abnormalities are evident because tubular disease follows glomerular dysfunction in the usual course of renal disease.

(b) Urine urea/plasma urea and urine creatinine/plasma creatinine ratios are decreased.

(c) BUN concentration is moderately increased in renal failure in the horse because of intestinal excretion of urea. Very high BUN concentrations suggest prerenal complications.

(d) BUN concentration does not increase proportionally to creatinine concentration in the ruminant because of excretion of urea via the rumen.

(e) Single BUN determinations are not reliable prognostic indicators. Evaluation of serial BUN concentrations provides better monitoring of disease progression and more accurate clinical prognosis.

(3) Pre-renal and renal azotemia often coexist.

c. Postrenal azotemia (obstruction or postrenal leakage) (Case 20)

(1) Clinical signs of postrenal azotemia include oliguria and anuria. Physical findings and/or radiologic or ultrasound examinations usually are sufficient to diagnose postrenal azotemia.

(2) The urine specific gravity is variable; concentration abnormalities may or may not occur.

(3) BUN concentration should return to the reference interval several days after relief of the urinary tract obstruction or repair of a rupture involving the ureter, bladder, or urethra.

d. Interpretation of BUN in birds

(1) Determination of BUN concentration is used as a sensitive indicator of dehydration in birds.

(2) The small amount of urea in avian blood precludes its use as a measure of renal function; however, there is a correlation between renal failure and BUN concentration in the pigeon.

(3) Uric acid is the end product of nitrogen catabolism in birds. Increased serum or plasma uric acid concentration (hyperuricemia) is the best indicator of potential renal disease in birds.

Free ebooks ==> www.Ebook777.com

5. Causes of decreased BUN concentration
   a. Low BUN values may be seen in hepatic insufficiency (decreased functional mass) (Cases 12, 13), low protein diets, and following the administration of anabolic steroids.
   b. Possible mechanisms resulting in a decreased BUN concentration include decreased production of urea via decreased hepatic urea cycle function or reduced protein catabolism and availability of ammonia for urea synthesis.
   c. Young animals may have low BUN values from increased fluid intake, increased urine output, and a high anabolic state of rapid growth.

C. Serum creatinine
1. Basic concepts of metabolism
   a. Small quantities of creatinine may be absorbed when diets contain muscle. Intra-individual variations in serum creatinine concentrations are partially due to diet (i.e., amount of meat consumed).
   b. Most creatinine originates endogenously from nonenzymatic conversion of creatine that stores energy in muscle as phosphocreatine.
   c. A rather constant amount of creatine is converted to creatinine daily; creatinine is not reutilized.
   d. The creatine pool is influenced by muscle mass and disease.
      (1) Decreased serum creatinine concentration may accompany muscle disease and generalized wasting.
      (2) Increased serum creatinine concentration may result from conditioning (training) or rhabdomyolysis.
      (3) Most of the individual variation of creatinine values in healthy animals results from differences in muscle mass.
      (4) Males typically have higher creatinine values than females.
   e. Creatinine is distributed throughout the body water but diffuses slower than urea. Approximately four hours are required for equilibration of creatinine concentration (vs. one and a half hours for urea). In the case of ruptured urinary bladder, abdominal fluid creatinine concentrations are much higher than serum creatinine concentrations. This disparity in creatinine concentration persists longer than that of urea. Thus, differences in abdominal fluid and plasma creatinine concentrations are more useful diagnostically than urea concentrations.
2. Creatinine excretion
   a. Renal excretion
      (1) Creatinine is freely filtered by the glomerulus. Tubular reabsorption does not occur.
      (2) A small amount of creatinine is secreted by the proximal tubules of male dogs.
      (3) Serum creatinine is a more accurate measurement of GFR than BUN because of the lack of tubular reabsorption and minimal tubular secretion.
   b. Gastrointestinal
      (1) A small amount of creatinine is excreted by this route.
      (2) Excreted creatinine is primarily degraded by enteric organisms, but minor recycling probably occurs.
3. Interpretation of increased serum creatinine concentration
   a. Creatinine concentration is not significantly affected by diet or catabolic factors but it is affected by muscle mass.
   b. A reduced GFR affects creatinine in a similar manner to BUN (Case 14).
   c. Creatinine determination provides information similar to that of BUN in renal disease as well as postrenal obstruction or leakage (Cases 10, 18, 19, 20, 22).
   d. Creatinine, like BUN, has relatively poor sensitivity in the diagnosis of renal disease in the dog and cat. Three-fourths of renal function must be lost before abnormalities in creatinine concentration can be discerned.

www.Ebook777.com

e. Creatinine concentration is a more sensitive indicator of renal disease than BUN concentration in the cow and horse. In these species, the potential for gastrointestinal excretion of creatinine is limited in contrast to urea (Case 3).

f. Diuresis and dialysis have less effect on lowering serum creatinine concentration than on lowering BUN concentration. Urine flow rate has less effect on creatinine concentration than on BUN concentration because creatinine diffuses more slowly between fluid compartments than BUN. Therefore, diuresis and dialysis promote greater excretion of urea than creatinine.

g. Noncreatinine chromagens may result in false-high test values in serum with most clinical assays. Ketones are the most significant of these interfering compounds.

h. Urine creatinine/serum creatinine ratios have been advocated to distinguish pre-renal azotemia from renal azotemia, but are of questionable diagnostic value.

i. Serum BUN/creatinine ratio

(1) This ratio has been suggested to be of value in the differential diagnosis of azotemia because of differences in tubular reabsorption and diffusion rates and because of the effects of diet and protein metabolism on the two compounds.

(2) Clinical experience has shown that there are too many variables for the serum BUN/creatinine ratio to be of use as a diagnostic parameter in veterinary medicine, particularly in dogs.

j. Creatinine concentration is considered to be very insensitive and a poor diagnostic test in birds. However, elevated creatinine concentrations have been reported in renal disease, egg-related coelomitis, and sepsis.

D. Uric acid

1. Uric acid is the major component of nitrogen catabolism in birds.

2. Hyperuricemia is a good indicator of renal disease in birds.

a. Some avian species can compensate for renal damage before elevations in serum uric acid concentration are observed. This limits the sensitivity of uric acid determination in some birds.

b. Hyperuricemia may occur during ovulation and after eating, limiting the test specificity in detecting renal disease.

3. Normouricemia does not exclude renal disease.

4. Juvenile birds have lower uric acid concentrations than adults. Birds consuming a diet of grain have lower concentrations of uric acid than carnivorous species.

E. Measurement of GFR. The ideal substance for measuring GFR should be exclusively cleared by glomerular filtration and neither reabsorbed nor secreted by the renal tubules. It also should not be subject to metabolism or excretion by extrarenal routes. All tests of GFR are affected by decreased renal perfusion.

1. Endogenous creatinine clearance

a. The volume of urine formed during a known period of time is measured. Concurrent urine and serum creatinine concentrations are determined at the beginning and end of the time period. The endogenous creatinine clearance is calculated as follows:

(1) Creatinine clearance = [urine creatinine (mg/dL) × urine volume (mL/min) ÷ serum creatinine (mg/dL)] ÷ body weight (kg).

(2) The results are expressed as mL/min/kg.

b. Endogenous creatinine clearance is used as a measure of glomerular filtration rate because blood creatinine concentrations are rather constant. Most creatinine is filtered by the glomerulus; tubular reabsorption does not occur.

c. Inaccurate estimation of the GFR is related to tubular secretion of creatinine, extra-renal excretion of creatinine, and measurement of noncreatinine chromagens.

2. Exogenous creatinine and isohexol clearance

a. These compounds are administered to the patient and clearance is measured as outlined above.

Free ebooks ==> www.Ebook777.com

3. Inulin clearance for measuring GFR and *p*-aminohippuric acid clearance for measuring renal plasma flow, respectively, are more accurate tests but are available only at large research centers.

F. Urine electrolyte clearance ratios (fractional clearances)

1. Fractional clearance or excretion (FE) expresses the proportion of a substance that is excreted in the urine compared with that filtered through the glomerulus. Glomerular concentration is equivalent to serum concentration of the analyte. Dietary intake and intestinal absorption of the material affect interpretation of resultant values.

2. Electrolyte clearance may be quantified by comparison to the clearance of endogenous creatinine (% creatinine clearance).

a. This procedure simultaneously measures the electrolyte and creatinine concentrations in single urine and serum samples ("spot" measurements), allowing determination of electrolyte clearance without knowledge of urine flow rate or volume.

b. The formula to calculate electrolyte clearance is

$$FE = (\text{urine electrolyte/serum electrolyte}) \div (\text{urine creatinine/serum creatinine}) \times 100.$$

3. Fractional clearance of sodium (FE_{Na}) is most commonly used.

a. FE_{Na} is increased (greater than 1%) in tubular failure of any kind; however, clearance values can be within the reference interval or decreased in acute glomerular disease.

b. FE_{Na} is decreased (less than 1%) in prerenal azotemia. It may be greater than 1% in animals with prerenal azotemia that are receiving diuretics and in animals with renal azotemia.

4. Fractional clearance of potassium (FE_K)

a. Clinical use of FE_K is controversial; values do not correlate well with 24-hour urine potassium concentration.

b. Hypokalemia with a FE_K of less than 6% is considered by some to represent nonrenal loss, while a FE_K of greater than 6% is due to renal loss.

c. The clinical usefulness of evaluating potassium disorders by comparison of urine and serum potassium concentrations, with and without aldosterone activity (transtubular potassium gradient), is uncertain.

5. Fractional clearance of phosphorus (FE_P) also is increased in renal failure, provided that oliguria or anuria are not present.

6. Fractional excretion of bicarbonate (FE_{HCO3^-}) is useful in human medicine for the classification of renal tubular acidosis. The FE_{HCO3^-} is less than 5% to 10% in Type I RTA, greater than 15% in Type II RTA, and 5% to 15% in Type IV RTA.

7. Variability in intake and nonurinary excretion of electrolytes results in variability of the clearance ratios in healthy animals. These factors have limited the usefulness of electrolyte fractional excretion to detect renal disease. Also, some laboratory instruments cannot quantitate urinary electrolytes.

G. Miscellaneous alterations occurring in renal disease

1. Progressive nonregenerative anemia occurs in chronic renal disease (Case 15).

a. The mechanism of the anemia is multifactorial.

b. The major factor is reduced erythropoietin secretion because of decreased functional renal parenchyma.

c. Hemorrhage, decreased erythrocyte lifespan, and bone marrow suppression by uremic toxins are contributing factors.

2. Hyperphosphatemia is associated with decreased GFR (Cases 15, 17, 18, 19, 20).

a. Hyperphosphatemia is less significant in the cow because the kidney is not the major excretory route for phosphorus.

b. Hypophosphatemia may be observed in horses with decreased GFR.

3. Hyperkalemia occurs when oliguria or anuria is associated with acidosis in renal failure (Case 20).

www.Ebook777.com

4. Hypermagnesemia occurs in monogastric animals.
5. Polyuric renal failure can cause hypokalemia, especially in the cat and cow, but not in the horse.
6. Metabolic (titration) acidosis frequently is associated with renal failure in the dog and cat. The anion gap is increased due to uremic acids (Cases 15, 18).
7. Cows have a normal acid-base status or metabolic alkalosis because of rumen atony and HCl sequestration. However, the anion gap will be high due to salts of uremic acids. Paradoxical aciduria may occur when alkalosis, hypochloridemia, and hyponatremia coexist (Case 23).
8. Hypercalcemia is common in horses with renal disease because the kidney is the major excretory route of calcium.
9. Hypocalcemia is usually observed in cattle with renal disease (Case 22), whereas normocalcemia or slight hypocalcemia are the rule in dogs and cats (Cases 18, 19). Hypercalcemia occasionally occurs in dogs with renal disease.
10. Hyponatremia and hypochloridemia may occur with renal disease, particularly in the horse and cow (Cases 22, 23). The FE_{Na} is increased due to tubular failure and sodium is lost into the urine.
11. In primary glomerular disease, severe proteinuria results in hypoproteinemia from hypoalbuminemia (low A/G ratio). The tetrad of proteinuria (albuminuria), hypoproteinemia (hypoalbuminemia), edema, and hypercholesterolemia (hyperlipidemia) is referred to as "nephrotic syndrome" (Case 19).
12. Some of the highest plasma fibrinogen concentrations are observed in bovine renal failure. Fibrinogen values determined by heat precipitation may exceed 1,800 mg/dL (Case 22).
13. Hyperamylasemia and hyperlipasemia may be associated with renal failure in the dog because these enzymes are degraded and excreted by the kidney (Case 14).

REFERENCES

Adams LG, Polzin DJ, Osborne CA, et al: 1992. Correlation of urine protein/creatinine ratio and twenty-four-hour urinary protein excretion in normal cats and cats with surgically induced chronic renal failure. *J Vet Intern Med* 6:36–40.

Allen TA, Jones RI, Pervance J: 1987. Microbiologic evaluation of canine urine: Direct microscopic examination and preservation of specimen quality for culture. *J Am Vet Med Assoc* 190:1289–1291.

Bagley RS, Center SA, Lewis RM, et al: 1991. The effect of experimental cystitis and iatrogenic blood contamination on the urine protein/creatinine ratio in the dog. *J Vet Intern Med* 5:66–70.

Barsanti JA, Lees GE, Willard MD, Green RA: 1999. Urinary Disorders. *In:* Willard MD, Tvedten H, Turnwald GH (eds): *Small Animal Clinical Diagnosis by Laboratory Methods*, 3rd ed. W.B. Saunders Co., Philadelphia, pp. 108–135.

Bayly WM, Brobst DF, Elfers RS, et al: 1986. Serum and urinary biochemistry and enzyme changes in ponies with acute renal failure. *Cornell Vet* 76:306–316.

Berry WL, Leisewitz AL: 1996. Multifocal *Aspergillus terreus* discospondylitis in two German shepherd dogs. *J S Afr Vet Assoc* 67:222–228.

Bertone JJ, Traub-Dargatz JL, Fettman MJ, et al: 1987. Monitoring the progression of renal failure in a horse with polycystic kidney disease: Use of the reciprocal of serum creatinine concentration and sodium sulfanilate clearance half-time. *J Am Vet Med Assoc* 191:565–568.

Biewenga WJ: 1986. Proteinuria in the dog: A clinicopathological study in 51 proteinuric dogs. *Res Vet Sci* 4:257–264.

Billet J-PHG, Moore AH, Holt PE: 2002. Evaluation of a bladder tumor antigen test for the diagnosis of lower urinary tract malignancies in dogs. *Am J Vet Res* 63:370–373.

Free ebooks ==> www.Ebook777.com

Borjesson DL, Christopher MM, Ling GV: 1999. Detection of canine transitional cell carcinoma using a bladder tumor antigen urine dipstick test. *Vet Clin Pathol* 28:33–38.

Brobst D: 1989. Urinalysis and associated laboratory procedures. *Vet Clin North Am Small Anim Pract* 19:929–949.

Center SA, Smith CA, Wilkinson E, et al: 1987. Clinicopathologic, renal immunofluorescent, and light microscopic features of glomerulonephritis in the dog: 41 cases (1975–1985). *J Am Vet Med Assoc* 190:81–90.

Center SA, Wilkinson E, Smith CA, et al: 1985. 24-hour urine protein/creatinine ratio in dogs with protein-losing nephropathies. *J Am Vet Med Assoc* 187:820–824.

Chew DJ, DiBartola SP: 1998. *Interpretation of Canine and Feline Urinalysis*. Ralston Purina Company, St. Louis, MO.

DiBartola SP, De Morais HA: 2000. Disorders of Potassium: Hypokalemia and Hyperkalemia. *In:* DiBartola SP (ed): *Fluid Therapy in Small Animal Practice*, 2nd ed. W.B. Saunders Co., Philadelphia, pp. 91–93.

DiBartola SP, Green RA, De Morais HA, et al: 1999. Electrolyte and Acid-Base Disorders. *In:* Willard MD, Tvedten H, Turnwald GH (eds): *Small Animal Clinical Diagnosis by Laboratory Methods*, 3rd ed. W.B. Saunders Co., Philadelphia, pp. 97, 100.

DiBartola SP, Rutgers HC, Zack PM, et al: 1987. Clinicopathologic findings associated with chronic renal disease in cats: 74 cases (1973–1984). *J Am Vet Med Assoc* 190:1196–1202.

DiBartola SP, Tarr MJ, Parker AT, et al: 1989. Clinicopathologic findings in dogs with renal amyloidosis: 59 cases (1976–1986). *J Am Vet Med Assoc* 195:358–364.

Echols S: 1999. Collecting diagnostic samples in avian patients. *Vet Clin North Am Exot Anim Pract* 2:636–637.

Elliot J, Barber PJ: 1998. Feline chronic renal failure: Clinical findings in 80 cases diagnosed between 1992 and 1995. *J Small Anim Pract* 39:78–85.

Fettman MJ: 1987. Evaluation of the usefulness of routine microscopy in canine urinalysis. *J Am Vet Med Assoc* 190:892–896.

Fettman MJ: 1989. Comparison of urinary protein concentration and protein/creatinine ratio vs. routine microscopy in urinalysis of dogs: 500 cases (1987–1988). *J Am Vet Med Assoc* 195:972–976.

Finco DR, Brown SA, Barsanti JA, et al: 1997. Reliability of using random urine samples for "spot" determination of fractional excretion of electrolytes in cats. *Am J Vet Res* 58:1184–1187.

Finco DR: 1997. Kidney Function. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 5th ed. Academic Press, San Diego, pp. 441–484.

Finco DR, Tabaru H, Brown SA, et al: 1993. Endogenous creatinine clearance measurement of glomerular filtration rate in dogs. *Am J Vet Res* 54:1575–1578.

Garry F, Chew DJ, Hoffsis GF: 1990. Enzymuria as an index of renal damage in sheep with induced aminoglycoside nephrotoxicosis. *Am J Vet Res* 51:428–432.

Grauer GF, Greco DS, Behrend EN, et al: 1995. Estimation of quantitative enzymuria in dogs with gentamycin-induced nephrotoxicosis using urine enzyme creatinine ratios from spot urine samples. *J Vet Intern Med* 9:324–327.

Holan KM, Kruger JM, Gibbons SN, et al: 1997. Clinical evaluation of a leukocyte esterase test-strip for detection of feline pyuria. *Vet Clin Pathol* 26:126–131.

www.Ebook777.com

Hughes D: 1992. Polyuria and polydipsia. *Compend Contin Educ Pract Vet* 14:1161.

Kohn LW, Chew DJ: 1987. Laboratory diagnosis and characterizations of renal disease in horses. *Vet Clin North Am Equine Pract* 3:585–615.

Lulich JP, Osborne CA: 1990. Interpretation of urine protein-creatinine ratios in dogs with glomerular and nonglomerular disorders. *Compend Contin Educ Pract Vet* 12:59–72.

Lulich JP, Osborne CA, O'Brien TD, et al: 1992. Feline renal failure: Questions, answers, questions. *Compend Contin Educ Pract Vet* 14:127–152.

McBride LJ: 1998. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Lippincott Williams and Wilkins, Philadelphia.

Moore FM, Brum SL, Brown L: 1991. Urine protein determination in dogs and cats: Comparison of dipstick and sulfosalicylic acid procedures. *Vet Clin Pathol* 20: 95–97.

Murgier P, Jakins A, Bexfield N, et al: 2009. Comparison of semiquantitative test strips, urine protein electrophoresis, and an immunoturbidimetric assay for measuring microalbuminuria in dogs. *Vet Clin Pathol* 38:485–492.

Neiger RD, Hagemoser WA: 1985. Renal percent clearance ratios in cattle. *Vet Clin Pathol* 14:31–35.

Newman DJ, Pugia MJ, Lott JA, et al: 2000. Urinary protein and albumin excretion corrected by creatinine and specific gravity. *Clinica Chimica Acta* 294:139–155.

Osborne CA, Davis LS, Sanna J, et al: 1990. Identification and interpretation of crystalluria in domestic animals: A light and scanning electron microscopic study. *Vet Med* 85:18–37.

Palacio J, Liste F, Gascon M: 1997. Enzymuria as an index of renal damage in canine leishmaniasis. *Vet Rec* 140:477–480.

Penny MD, Oleesky DA: 1999. Renal tubular acidosis. *Ann Clin Biochem* 36:408–422.

Pressler BM, Vaden SL, Jensen WA, et al: 2008. Detection of canine microalbuminuria using semiquantitative test strips designated for use with human urine. *Vet Clin Pathol* 31:56–60.

Pugia MJ, Lott JA, Profitt JA, et al: 1999. High-sensitivity dye binding assay for albumin in urine. *J Clin Lab Anal* 13:180–187.

Ritchie BW, Harrison GJ, Harrison LR (eds): 1994. *Avian Medicine: Principles and Applications*. Wingers Publishing Company, Lake Worth, FL.

Ross DL, Neely AE: 1983. *Textbook of Urinalysis and Body Fluids*. Appleton-Century-Crofts, Norwalk, CT.

Schultze AE, Jensen RK: 1989. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of canine urinary proteins for the analysis and differentiation of tubular and glomerular diseases. *Vet Clin Pathol* 18:93–97.

Welles EG, Whatley EM, Hall AS, et al: 2006. Comparison of Multistix PRO dipsticks with other biochemical assays for determining urine protein (UP), urine creatinine (UC), and UP:UC ratio in dogs and cats. *Vet Clin Pathol* 35:31–36.

Wallace JF, Pugia MJ, Lott JA, et al: 2001. Multisite evaluation of a new dipstick for albumin, protein, and creatinine. *J Clin Lab Analysis* 15:231–235.

Free ebooks ==> www.Ebook777.com

CHAPTER 10

MUSCLE

Robert L. Hall, DVM, PhD, and Holly S. Bender, DVM, PhD

Muscle diseases characterized by degeneration, necrosis, or inflammation with degeneration/necrosis may be detected with clinical chemistry techniques. The common feature of these conditions is disruption of muscle cell membranes and release of enzymes and cytoplasmic contents into surrounding blood and lymph. Muscular atrophy and neoplastic conditions not associated with cell membrane disruption usually do not cause changes in standard clinical chemistry tests.

I. SERUM ENZYMES OF MUSCLE ORIGIN

A. Creatine kinase (CK)

1. CK is critical to muscle energy production. CK makes adenosine triphosphate (ATP) available for muscle contraction by catalyzing the transfer of a high-energy phosphate bond from creatine phosphate to adenosine diphosphate (ADP). CK also catalyzes the reverse reaction when muscles are at rest. Muscle cells contain eight times more creatine phosphate than ATP, thereby providing a reservoir of high-energy phosphate bonds for contraction.

2. CK is primarily a cytosolic enzyme with highest activity in skeletal muscle, cardiac muscle, and brain. Liver has negligible CK activity.

3. CK is one of the most organ-specific clinical enzymes, as most serum CK activity is of muscle origin.

4. CK is a dimeric enzyme with two subunits, designated B for brain and M for muscle. Three principal isoenzyme types exist: CK-BB (CK_1), CK-MB (CK_2), and CK-MM (CK_3).

a. CK isoenzymes can be separated electrophoretically, and the proportion of each determined. CK-BB is the most anodal.

b. CK isoenzymes may also be separated by species-specific immunological methods or by ion-exchange chromatography.

c. CK-BB is present in brain, peripheral nerves, cerebrospinal fluid, and viscera.

d. CK-MB is present in cardiac muscle with relatively low activity in other tissues.

e. CK-MM is present in both skeletal and cardiac muscle.

f. CK activity in serum is mostly CK-MM, followed by CK-BB, and very little, if any, CK-MB.

g. CK isoenzyme analysis has not been very informative in veterinary medicine and is rarely used in a clinical setting.

5. Erythrocytes contain very little CK, but enzymes and intermediates released from erythrocytes may affect assay reactions, causing falsely increased activity when hemolysis is present. Hemolyzed serum specimens are unsatisfactory for determination of CK activity.

6. Dilution of serum samples to reduce CK activity to an acceptable range for measurement may paradoxically falsely increase enzymatic activity due to dilution of naturally occurring CK inhibitors.

www.Ebook777.com

7. Serum CK activity in healthy dogs varies with age and breed.
   a. CK activity decreases with age; pups can have much higher CK activity than adult dogs.
   b. Adult levels of CK activity are achieved by seven to 12 months of age.
   c. Small breeds of dogs tend to have higher activities.
8. Plasma CK half-life is short (less than three hours in dogs, approximately four hours in cattle, less than two hours in horses).
9. Specimens analyzed for CK activity should be processed quickly.
   a. Reports vary concerning stability of CK activity in serum and plasma.
   b. CK activity may diminish if a prolonged delay occurs between obtaining and analyzing the serum specimen (e.g., transport via mail from the veterinary hospital to the laboratory).
   c. If CK analysis must be delayed beyond 12 hours, serum or plasma should be frozen (−20°C) to minimize loss of activity.
   d. Loss of activity can be partially reversed through the use of reducing agents in the assay system.
   e. Serum CK activity is higher than plasma CK activity in the dog, and perhaps other species, due to release of CK from platelets during clot formation.

B. Aspartate aminotransferase (AST) (Chapter 7)
1. AST catalyzes the reversible transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. Oxaloacetate can enter the Krebs cycle. The enzyme was previously known as serum glutamic oxaloacetic transaminase (SGOT).
2. AST has cytosolic and mitochondrial isoenzymes, and AST activity is present in almost all cells, including red blood cells.
3. Serum AST activity is tissue-nonspecific, but muscle and liver are the major sources.
4. Plasma AST half-life is less than 12 hours in cats, approximately 12 hours in dogs, approximately 18 hours in swine, and probably longer in horses and cattle.
5. Plasma AST half-life is longer than plasma CK half-life.
6. AST is relatively stable at room, refrigerator, and freezer temperatures.
7. Separate serum or plasma from cells immediately because imperceptible hemolysis may falsely increase AST activity.

C. Alanine aminotransferase (ALT) (Chapter 7)
1. ALT catalyzes the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and glutamate. Pyruvate can be used for gluconeogenesis or enter the Krebs cycle. The enzyme was previously known as serum glutamic pyruvic transaminase (SGPT).
2. ALT is primarily a cytosolic enzyme considered liver-specific in dog and cat; however, increases in ALT activity have been reported with muscle diseases such as X-linked muscular dystrophy or toxic myopathies.
3. ALT has been used as a muscle-specific enzyme in large animals because hepatic ALT activity in large animals is very low. Increased activity has been reported in myopathies of lambs, pigs, and horses.
4. Plasma ALT half-life in dogs is considered approximately two and a half days. Plasma ALT half-life is likely greater than that for AST or CK in most species.

D. Lactate dehydrogenase (LDH)
1. LDH is a cytosolic enzyme present in all cells, and therefore all tissues, that catalyzes the reversible conversion of L-lactate to pyruvate.
2. Muscle, liver, and erythrocytes are usually the sources of high LDH activity in serum.
3. LDH is less useful than CK and AST for determining muscle damage because it lacks tissue specificity and is significantly affected by even mild hemolysis.
4. LDH is a tetrameric enzyme made up of two subunits, H and M, to form five isoenzymes: LDH_1 (H4), LDH_2 (H3M1), LDH_3 (H2M2), LDH_4 (H1M3), and LDH_5 (M4). In general, isoenzymes with

Free ebooks ==> www.Ebook777.com

mostly H subunits (LDH_1 and LDH_2) predominate in aerobic tissue and those with mostly M subunits predominate in anaerobic tissue.

a. LDH isoenzymes can be separated electrophoretically, and the proportion of each determined. LDH_1 (H4) is the most anodal.

b. LDH_1 (H4) is heat stable; $LDH_{2–5}$ are inactivated in serum when heated at 65°C for 30 minutes.

c. LDH_1 (H4) is the principal isoenzyme in cardiac muscle and kidney.

d. LDH_5 (M4) is the principal isoenzyme in skeletal muscle and erythrocytes.

e. Liver contains primarily LDH_4 and LDH_5 (H1M3 and M4) in many species, but the liver isoenzyme profile for cattle and sheep is more similar to heart.

f. All tissues contain variable amounts of the five LDH isoenzymes, and even with electrophoretic separation, the tissue source of increased serum LDH activity is often uncertain.

5. Separate serum or plasma from cells immediately because imperceptible hemolysis may falsely increase LDH activity.

6. LDH is unstable at freezer temperatures (−20°C), and more stable at refrigerator temperatures (4°C).

7. Plasma LDH half-life varies for each isoenzyme, with LDH_1 (H4) the longest and LDH_5 (M4) the shortest.

E. Aldolase

1. Aldolase in muscle (also called aldolase A) is a cytosolic enzyme that catalyzes the cleaving of fructose-1,6-bisphosphate to form glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the glycolytic utilization of fructose for energy.

2. Aldolase has been used to investigate skeletal muscle disorders, but isoenzymes exist in multiple tissues, including liver and heart.

3. Aldolase is generally considered inferior to CK for diagnosis of skeletal muscle disorders because CK has better diagnostic sensitivity and greater ease of measurement in the laboratory.

II. DIAGNOSTIC SIGNIFICANCE OF CK, AST, AND LDH

A. Increased serum CK, AST, and LDH activities occur with degenerative or necrotizing muscle injury. Diseases associated with increased serum activity of these enzymes are listed in Table 10.1.

1. CK is the most sensitive serum enzyme indicator of striated muscle damage.

a. CK is the enzyme of choice to detect skeletal muscle damage.

b. Serum CK activity increases within four to six hours after muscle injury, and typically reaches maximum levels in six to 12 hours.

c. Serum CK activity returns to the reference interval within 48 to 72 hours once muscle injury abates.

d. Persistently high serum CK activity indicates continued muscle injury.

e. The magnitude of increased serum CK activity generally correlates with the extent of muscle injury, but exceptions occur. Only marked (e.g., greater than 5,000 IU/L) or moderate but persistent increases (e.g., greater than 2,000 IU/L) are considered clinically significant.

f. Minor increases of serum CK activity are considered more significant in cats because of smaller muscle mass and comparatively low CK activity in cat muscle. However, anorexic cats can exhibit increased serum CK activity in the absence of diseases directly affecting muscle.

2. Serum AST activity increases slower than serum activities of CK and LDH following muscle injury; increased serum AST activity may persist several days after muscle injury abates.

3. Serum LDH activity increases following muscle injury are less apparent than those for CK and AST and more difficult to assess because of the broad tissue distribution of LDH.

B. Serum CK and LDH isoenzymes have some tissue specificity.

www.Ebook777.com

TABLE 10.1

DISEASES WITH HIGH SERUM ENZYME ACTIVITY (CK, LDH, AST) OF MUSCLE ORIGIN. CONDITIONS WITH SEVERE DISRUPTION OF MUSCLE CELL MEMBRANES MAY PRESENT WITH MYOGLOBINURIA.

- Inflammatory myopathies
  - Infectious
    - Bacterial
      - Clostridial myositis
      - Immune-mediated *Streptococcus equi* infections
      - *Leptospira icterohaemorrhagiae*
      - *Pasteurella multocida* myositis (cats)
      - Staphylococcal and streptococcal myositis (dogs)
    - Parasitic
      - *Hepatozoon canis*
      - *Neospora caninum*
      - *Otobius megnini* (equine ear tick)
    - Associated muscle cramping
      - *Sarcocystis* spp.
      - *Toxoplasma gondii*
      - *Trypanosoma cruzi*
    - Viral
      - Blue tongue
      - Bovine ephemeral fever
      - Bovine virus diarrhea
      - Equine herpesvirus 1
      - Equine infectious anemia
      - Equine influenza virus A2
      - Malignant catarrhal fever
  - Noninfectious
    - Dermatomyositis (dogs)
    - Eosinophilic myositis (dogs, cattle)
    - Immune-mediated polymyositis (dogs, cats)
    - Masticatory muscle myositis (dogs)
- Traumatic myopathies
  - Accidental
  - Capture myopathy (birds)
  - CNS disease (especially with seizures)
  - Extreme exercise
  - Post-operative
  - Post-cardiac resuscitation
  - Prolonged recumbency of large animals (downer animals)
  - Intramuscular injections of irritating substances
  - Gastrocnemius rupture (horses)
  - Secondary to joint diseases
  - Tourniquet syndrome
- Degenerative myopathies
  - Inherited or congenital
    - Lysosomal alpha-1,4-glucosidase deficiency (dogs, Shorthorn and Brahman cattle)
    - Debranching enzyme deficiency (dogs, foals)
    - Hyperkalemic periodic paralysis (horses)
    - Mitochondrial myopathy
      - Decreased cytochrome C oxidase (Sheep Dogs)
      - Respiratory chain defect (Arabian horses)
    - Muscular dystrophy (cattle, sheep, dogs, cats, chickens)
    - Myophosphorylase deficiency (cattle)
    - Myotonia (dogs, goats, horses)
      - Chloride-channel myotonia (goats)
    - Phosphofructokinase deficiency (English Springer Spaniels)
    - Lysosomal alpha-polysaccharide storage myopathy (horses)
  - Metabolic or unknown cause
    - Acquired equine motor neuron disease
    - Anesthesia (horses, swine)
    - Canine exertional rhabdomyolysis in racing Greyhounds
    - Capture myopathy
    - Equine rhabdomyolysis (paralytic myoglobinuria, azoturia, Monday morning disease, tying-up syndrome)
    - Hyperadrenocorticism (dogs, horses)
    - Hypokalemic polymyopathy (cats)
    - Hypothyroidism (dogs, horses)
    - Malignant hyperthermia (swine, dogs)
    - Porcine stress syndrome
  - Toxic
    - Blister beetle toxicosis
    - Bracken fern (myocardial necrosis in horses)
    - *Cassia* sp. (coffee weed, castor bean, sicklepod) toxicosis (cattle, horse)
    - Copper poisoning (sheep)
    - Gossypol toxicosis (cattle, horse)
    - Horsetail (*Equisetum arvense*) (horses)
    - Ionophore-induced myocardial and skeletal muscle degeneration from monensin, lasalocid, maduramicin, or salinomysin (horse, ruminants, turkey)
    - Organophosphate toxicosis
  - Nutritional
    - Anorexia (cats)
    - Thiamine (vitamin B_1) deficiency
    - Destruction of thiamine in heat (above 100°C) processed diets (dogs, cats)
      - Excessive thiaminase ingestion
      - Bracken fern (horses)
      - Vitamin E/selenium deficiency (calves, lambs, yearling cattle, foals, kids, swine, dogs, ostriches)
      - Horsetail (*Equisetum arvense*) (horses)
  - Ischemic myopathies
    - Aortic thrombosis (cats)
    - Bacterial endocarditis
    - Dirofilariasis
    - Iliac thrombosis (horses, calves)

Free ebooks ==> www.Ebook777.com

1. Serum CK-MM and LDH5 (M4) activities increase in greater proportion following skeletal muscle injury.
2. Serum CK-MB and LDH1 (H4) activities increase in greater proportion following cardiac muscle injury. However, CK-MB has not proven effective for diagnosing equine cardiac muscle injury.
3. Serum LDH1 (H4) activity increases in hemolytic disease but also hemolyzed samples.
4. In general, determination of serum CK and LDH isoenzyme patterns have not proven valuable or practical for clinical veterinary medicine. Cardiac troponins are proving to be more sensitive, specific, and practical for assessing cardiac muscle injury.

C. Serum CK activity can be affected by minor muscle injuries unrelated to primary muscle disease.
1. Placement of electrodes for electromyography increases serum CK activity but usually not above the reference interval.
2. Intramuscular injections increase serum CK activity. Irritating drugs (e.g., ketamine) or drug vehicles can cause dramatic increases lasting up to one week.
3. Traumatic venipuncture, even in the absence of hemolysis, can increase serum CK activity. Sample contamination with perivenous connective tissue or muscle can occur.
4. Strenuous exercise in dogs and horses increases serum CK and LDH activities.
   a. Rarely more than three-fold baseline after light exercise
   b. Physical training minimizes post-exercise increases.
5. Animal shipping may cause increased serum CK activity.

D. Cerebrospinal fluid (CSF) CK activity originates from the brain and may increase with central nervous system (CNS) disease.
1. Increased CSF-CK activity does not affect serum CK activity.
2. Increased serum CK activity associated with CNS disease likely originates from injury to muscle cells during convulsions (e.g., involuntary muscle contractions or contusions) or prolonged recumbency.

E. Increased serum LDH activity has been associated with canine lymphoma.

F. Lymphoma in cattle is associated with high serum LDH activity in about 70% of affected animals.
1. Serum LDH activities overlap between clinically healthy and affected cattle.
2. Serum LDH activity is not elevated for cattle with persistent lymphocytosis.

G. CK, AST, and LDH activities are used to diagnose skeletal muscle disease in birds.
1. As in mammals, serum CK activity is the most muscle-specific indicator.
2. Hemolysis markedly increases avian serum LDH activity.

III. OTHER LABORATORY FINDINGS IN MUSCLE DISEASE

A. Troponins
1. Troponins are globular proteins bound to tropomyosin that help modulate the interaction between actin and myosin within the myofibril of striated muscle.
   a. Three troponin proteins form a regulatory complex: troponin I, troponin T, and troponin C.
   b. Troponin I and T have genetically distinct cardiac isoforms and are therefore useful for evaluating cardiac muscle injury.
   c. Cardiac troponins I and T (cTnI and cTnT) are considered the markers of choice for acute cardiac injury in humans, replacing CK-MB.
   d. cTnI and cTnT are highly conserved in mammalian species and many human immunoassays cross-react with cardiac troponins in animals.
   e. Many functional assay systems exist for cTnI. The cTnT assay is limited to one vendor and less available.
   f. There is no clear diagnostic advantage to measuring both cTnI and cTnT.

www.Ebook777.com

2. Cardiac troponins are released into blood by degenerating/necrotic cardiac muscle cells.
3. Serum cardiac troponin concentrations are normally very low, and increases are apparent within a few hours of cardiac muscle injury.
4. Cardiac troponins have short half-lives (hours) so serum levels decrease quickly (one or two days) unless cardiac muscle injury is ongoing.
5. Increased serum cardiac troponin concentrations have also been observed with strenuous exercise in horses and renal failure.

B. Natriuretic peptides

1. Two natriuretic peptides, atrial or A-type (ANP) and brain or B-type (BNP), have been studied as markers for cardiac muscle function/dysfunction.
2. Natriuretic peptides are released as prohormones from cardiac muscle in response to mechanical stress or cardiac muscle stretching, most often due to increased pressure.
3. The prohormones are cleaved into inactive N-terminal fragments (NTproANP or NTproBNP) and active C-terminal fragments (ANP and BNP).
4. Active ANP and BNP contribute to cardiovascular homeostasis by inhibiting the renin-angiotensin-aldosterone system, promoting vasodilatation, increasing natriuresis and diuresis, and decreasing arterial blood pressure.
5. Assays have been developed for the inactive N-terminal fragments and active C-terminal fragments.
   a. BNP and NTproBNP are used in human medicine as sensitive but nonspecific markers for congestive heart failure.
   b. Human BNP and NTproBNP assays do not cross-react well with animal peptides, so development of species-specific assays has limited use of these markers until recently.
   c. Half-lives for the inactive fragments may be longer than those for active fragments, making the inactive fragments (NTproANP and NTproBNP) more attractive as potential markers of heart disease.

C. Myoglobin

1. Myoglobin is a heme protein responsible for transporting and storing oxygen within muscle cells. Myoglobin is normally absent in serum.
2. Myoglobin is considered a specific and sensitive indicator of muscle necrosis.
   a. Myoglobin released from muscle enters blood immediately.
   b. CK and AST first enter lymph, delaying increased serum activity.
   c. Serum myoglobin falls rapidly once muscle injury abates.
3. Myoglobin is a low-molecular-weight monomer that, unlike hemoglobin, does not bind significantly to plasma proteins.
4. Myoglobin readily passes through the glomerulus, and plasma may not become discolored.
5. Both myoglobin and hemoglobin cause a positive urine occult blood reaction on urinalysis test strips (Chapter 9) and pink to red to brown urine depending on concentration and degradation/oxidation.
   a. An ammonium sulfate precipitation test to differentiate myoglobin from hemoglobin is unreliable (in theory, hemoglobin precipitates in an 80% ammonium sulfate solution, but myoglobin does not).
   b. Myoglobinuria is typically associated with other evidence of muscle injury, normal plasma color, and normal hematocrit.
   c. Hemoglobinuria is typically associated with other evidence of hemolysis (e.g., low hematocrit), pink to red plasma, and no supporting evidence for muscle injury.
6. Myoglobin can be measured in serum or urine by a variety of immunoassays but they are used infrequently in clinical veterinary medicine.

Free ebooks ==> www.Ebook777.com

D. Potassium

1. Intracellular fluid contains much more potassium than extracellular fluid.

2. Degeneration or necrosis of a large muscle mass may release enough potassium to cause hyperkalemia.

a. Correlation between hyperkalemia and increased muscle serum enzyme activity may be poor.

b. Hyperkalemia is more commonly associated with disorders of acid-base and electrolyte balance (Chapter 5).

3. Hyperkalemic periodic paralysis in Quarter horses is due to skeletal muscle sodium channel defects and often results in hyperkalemia.

a. Hyperkalemia is present during or immediately following clinical signs of disease (e.g., muscle fasciculations, collapse) but not between episodes.

b. Serum CK activity may be mildly increased or within the reference interval.

4. Hypokalemic polymyopathy may occur in cats with chronic renal failure or fed acidifying diets. Hypokalemia and increased serum CK activity are typical.

E. Lactate (Chapters 5 and 6)

1. Lactate is a byproduct of anaerobic glycolysis produced primarily by skeletal muscle, erythrocytes, brain, skin, and renal medulla.

2. Blood lactate concentration reflects a balance between lactate production, metabolism by the liver (used for gluconeogenesis), and elimination in urine.

3. Plasma lactate increases markedly after exercise in dogs with exercise-induced mitochondrial and lipid storage myopathies and Labrador Retrievers with hereditary myopathy.

4. Meticulous sample acquisition and handling are essential for lactate analysis in whole blood or plasma.

a. Blood should be collected into tubes containing sodium fluoride and potassium oxalate, chilled, and centrifuged within 15 minutes. Fluoride inhibits anaerobic glycolysis and lactate production by erythrocytes.

b. Blood lactate can increase if the patient struggles during venipuncture (muscle activity) or if the vein is held off for a prolonged period (venous stasis and local hypoxia).

c. Blood lactate also can increase after a meal.

F. Dystrophin and muscular dystrophy

1. Dystrophin is a cytoplasmic cytoskeleton protein that helps reinforce the sarcolemma and is part of a transmembrane protein complex that connects the muscle fiber to extracellular matrix.

2. An X-linked inherited deficiency of dystrophin causing muscular dystrophy has been described in dogs and cats. The disorder is characterized by progressive muscle degeneration/necrosis with hypertrophy of some muscle groups, and increased serum muscle enzyme activities.

3. Dystrophin in muscle can be assessed by immunocytochemistry or immunoblot techniques.

4. Cases of canine muscular dystrophy without dystrophin deficiency have been reported.

G. Acetylcholine receptor antibodies and myasthenia gravis

1. Immunoprecipitation tests are used to detect circulating autoantibodies to acetylcholine receptors in dogs and cats with immune-mediated myasthenia gravis.

2. Antibodies are absent in congenital (non-immune-mediated) myasthenia gravis.

H. Erythrocyte glutathione peroxidase activity and selenium deficiency

1. Decreased erythrocyte glutathione peroxidase activity is associated with selenium deficiency because selenium is a cofactor for the enzyme.

2. Serum vitamin E and selenium concentrations also can be measured.

I. Thiamine (vitamin B_1) deficiency

1. Although infrequent to rare, thiamine deficiency may cause myocardial necrosis as well as CNS disease.

2. Increased serum CK activity may not be apparent.

www.Ebook777.com

REFERENCES

Aktas M, Auguste D, Concorder D, et al: 1994. Creatine kinase in dog plasma: Preanalytical factors of variation, reference values, and diagnostic significance. *Res Vet Sci* 56:30–36.

Apple FS, Henderson RA: 1999. Cardiac Function. *In:* Burtis CA, Ashwood ER (eds): *Tietz Textbook of Clinical Chemistry*. W.B. Saunders, Philadelphia, pp. 1178–1203.

Balogh N, Gaal T, Ribiczeyné PS, et al: 2001. Biochemical and antioxidant changes in plasma and erythrocytes of pentathlon horses before and after exercise. *Vet Clin Pathol* 30:214–218.

Barth AT, Kommers GD, Salles MS, et al: 1994. Coffee senna (*Senna occidentalis*) poisoning in cattle in Brazil. *Vet Human Toxicol* 36:541–545.

Bastianello SS, Fourie N, Prozesky L, et al: 1995. Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin. II. Macropathology and histopathology. *Onderstepoort J Vet Res* 62:5–18.

Bender HS: 2003. Muscle. *In:* Latimer KS, Mahaffey EA, Prasse KW (eds): *Duncan and Prasse's Veterinary Laboratory Medicine*: *Clinical Pathology*, 4th ed. Iowa State Press, Ames, IA, pp. 260–269.

Benson JE, Ensley SM, Carson TL, et al: 1998. Lasalocid toxicosis in neonatal calves. *J Vet Diagn Invest* 10:210–214.

Bjurstrom S, Carlsten J, Thoren-Tolling K, et al: 1995. Distribution and morphology of skeletal muscle lesions after experimental restraint stress in normal and stress-susceptible pigs. *Zentralbl Veterinarmed A* 42:575–587.

Blot S: 2000. Disorders of the Skeletal Muscles. *In:* Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine Diseases of the Dog and Cat*, 5th ed. W.B. Saunders Co., Philadelphia, pp. 684–690.

Boemo CM, Tucker JC, Huntington PJ, et al: 1991. Monensin toxicity in horses. An outbreak resulting in the deaths of ten horses. *Aust Equine Vet* 9:103–107.

Boyd JW: 1983. The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. *Vet Clin Pathol* 12:9–24.

Braund KG: 1997. Idiopathic and exogenous causes of myopathies in dogs and cats. *Vet Med* 92:629–634.

Brown C: 2002. Rhabdomyolysis. *In:* Brown C, Bertone J (eds): *The 5-Minute Veterinary Consult: Equine*, 1st ed. Lippincott Williams and Wilkins, Baltimore, pp. 926–929.

Cardinet GH: 1997. Skeletal Muscle Function. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 5th ed. Academic Press, San Diego, pp. 407–440.

Cavaliere MJ, Calore EE, Haraguchi M, et al: 1997. Mitochondrial myopathy in *Senna occidentalis*-seed-fed chicken. *Ecotoxicol Environ Safety* 37:181–185.

Child G: 2000. Myopathy. *In:* Tilley LP, Smith FWK (eds): *The 5-Minute Veterinary Consult: Canine and Feline*, 2nd ed. Lippincott Williams and Wilkins, Philadelphia, pp. 990–993.

Christopher MM, O'Neill S: 2000. Effect of specimen collection and storage on blood glucose and lactate concentrations in healthy, hyperthyroid and diabetic cats. *Vet Clin Pathol* 29:22–28.

Connolly DJ, Guitian J, Boswood A, et al: 2005. Serum troponin I levels in hyperthyroid cats before and after treatment with radioactive iodine. *J Feline Med Surg* 7:289–300.

Free ebooks ==> www.Ebook777.com

Cornelisse CJ, Schott HC, Olivier NB, et al: 2000. Concentration of cardiac troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. *J Am Vet Med Assoc* 217:231–235.

Daugschies A, Hintz J, Henning M, et al: 2000. Growth performance, meat quality and activities of glycolytic enzymes in the blood and muscle tissue of calves infected with Sarcocystis cruzi. *Vet Parasitol* 88:7–16.

De La Corte FD, Valberg SJ, MacLeay JM, et al: 1999. Glucose uptake in horses with polysaccharide storage myopathy. *Am J Vet Res* 60:458–462.

Divers TJ, Kraus MS, Jesty SA, et al: 2009. Clinical findings and serum cardiac troponin I concentrations in horses after intragastric administration of sodium monensin. *J Vet Diagn Invest* 21:338–343.

Duncan JR, Prasse KW, Mahaffey EA: 1994. *Veterinary Laboratory Medicine: Clinical Pathology*. Iowa State University Press, Ames, IA, pp. 184–187.

Eades SC, Bounous DI: 1997. *Laboratory Profiles of Equine Diseases*. Mosby, St. Louis.

Edwards CM, Belford CJ: 1995. Hypokalaemic polymyopathy in Burmese cats. *Aust Vet Pract* 25:58–60.

Fascetti AJ, Mauldin GE, Mauldin GN: 1997. Correlation between serum creatine kinase activities and anorexia in cats. *J Vet Intern Med* 11:9–13.

Fonfara S, Loureiro J, Swift S, et al: 2010. Cardiac troponin I as a marker for severity and prognosis of cardiac disease in dogs. *Vet J* 184:334–339.

Gaschen F, Gaschen L, Seiler G, et al: 1998. Lethal peracute rhabdomyolysis associated with stress and general anesthesia in three dystrophin-deficient cats. *Vet Pathol* 35:117–123.

Gunes V, Atalan G, Citil M, et al: 2008. Use of cardiac troponin kits for the qualitative determination of myocardial cell damage due to traumatic reticuloperitonitis in cattle. *Vet Record* 162:514–517.

Gunes V, Ozcan K, Citil M, et al: 2010. Detection of myocardial degeneration with point-of-care cardiac troponin assays and histopathology in lambs with white muscle disease. *Vet J* 184:376–378.

Haraguchi M, Gorniak SL, Calore EE, et al: 1998. Muscle degeneration in chicks caused by *Senna occidentalis* seeds. *Avian Pathol* 27:346–351.

Harris PA, Mayhew I: 1998. Musculoskeletal Disease. *In:* Reed SM, Bayly WM (eds): *Equine Internal Medicine*, 1st ed. Saunders, Philadelphia, pp. 371–426.

Hinchcliff KW, Shaw LC, Vukich NS, et al: 1998. Effect of distance traveled and speed of racing on body weight and serum enzyme activity of sled dogs competing in a long-distance race. *J Am Vet Med Assoc* 213:639–644.

Hoffmann WE, Solter PF, Wilson BW: 1999. Clinical Enzymology. *In:* Loeb WF, Quimby FW (eds): *The Clinical Chemistry of Laboratory Animals*, 2nd ed. Taylor and Francis, Philadelphia, pp. 399–454.

Holbrook TC, Birks EK, Sleeper MM, et al: 2006. Endurance exercise is associated with increased plasma cardiac troponin I in horses. *Equine Vet J Suppl* 36:27–31.

Holmgren N, Valberg S: 1992. Measurement of serum myoglobin concentrations in horses by immunodiffusion. *Am J Vet Res* 53:957–560.

Horie Y, Tsubaki M, Katou A, et al: 2008. Evaluation of NT-pro BNP and CT-ANP as markers of concentric hypertrophy in dogs with a model of compensated aortic stenosis. *J Vet Intern Med* 22:1118–1123.

Kramer JW, Hoffmann WE: 1997. Clinical Enzymology. *In:* Kaneko JJ, Harvey JW, Bruss ML (eds): *Clinical Biochemistry of Domestic Animals*, 5th ed. Academic Press, San Diego, pp. 303–325.

www.Ebook777.com

Kraus MS, Jesty SA, Gelzer AR, et al: 2010. Measurement of plasma cardiac troponin I concentration by use of a point-of-care analyzer in clinically normal horses and horses with experimentally induced cardiac disease. *Am J Vet Res* 71:55–59.

Lappin MR: 1998. Polysystemic Protozoal Diseases. *In:* Nelson RW, Couto CG (eds): *Small Animal Internal Medicine*, 2nd ed. Mosby, St. Louis, pp. 1313–1324.

Leonardi F, Passeri B, Fusari A, et al: 2008. Cardiac troponin I (cTnI) concentration in an ovine model of myocardial ischemia. *Res Vet Sci* 85:141–144.

Lewis HB, Rhodes DC: 1978. Effects of I.M. (intramuscular) injections on serum creatine phosphokinase (CPK) values in dogs (Diagnostic or prognostic importance). *Vet Clin Pathol* 7:11–12.

MacDonald KA, Kittleson MD, Munro C, et al: 2003. Brain natriuretic peptide concentration in dogs with heart disease and congestive heart failure. *J Vet Intern Med* 17:172–177.

MacLeay JM, Valberg SJ, Pagan JD, et al: 2000. Effect of ration and exercise on plasma creatine kinase activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis. *Am J Vet Res* 61:1390–1395.

Mellanby RJ, Henry JP, Cash R, et al: 2009. Serum cardiac troponin I concentrations in cattle with cardiac and noncardiac disorders. *J Vet Intern Med* 23:926–930.

Meyer DJ, Harvey JW: 1998. *Veterinary Laboratory Medicine: Interpretation and Diagnosis*. W.B. Saunders, Philadelphia, pp. 157–186.

Moss DW, Henderson RA: 1999. Clinical Enzymology. *In:* Burtis CA, Ashwood ER (eds): *Tietz Textbook of Clinical Chemistry*. W.B. Saunders, Philadelphia, pp. 617–721.

Mushi EZ, Isa JFW, Chabo RG, et al: 1998. Selenium-vitamin E responsive myopathy in farmed ostriches (*Struthio camelus*) in Botswana. *Avian Pathol* 27:326–328.

Naylor JM: 1994. Equine hyperkalemic periodic paralysis: Review and implications. *Can Vet J* 35:279–285.

Nostell K, Haggstrom J: 2008. Resting concentrations of cardiac troponin I in fit horses and effect of racing. *J Vet Cardiol* 10:105–109.

O'Brien PJ: 2008. Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity. *Toxicology* 245:206–218.

O'Brien PJ, Smith DE, Knechtel TJ, et al: 2006. Cardiac troponin I is a sensitive, specific biomarker of cardiac injury in laboratory animals. *Lab Anim* 40:153–171.

Oyama MA, Sisson DD, Solter PF: 2007. Prospective screening for occult cardiomyopathy in dogs by measurement of plasma atrial natriuretic peptide, B-type natriuretic peptide, and cardiac troponin I concentrations. *Am J Vet Res* 68:42–47.

Panciera RJ, Ewing SA, Mathew JS, et al: 1999. Canine hepatozoonosis: Comparison of lesions and parasites in skeletal muscle of dogs experimentally or naturally infected with *Hepatozoon americanum*. *Vet Parasitol* 82: 261–272.

Parent J: 1999. Neurologic Disorders. *In:* Willard MD, Tvedten H, Turnwald GH (eds): *Small Animal Clinical Diagnosis by Laboratory Methods*, 3rd ed. W.B. Saunders, Philadelphia, pp. 279–287.

Philbey AW: 1991. Skeletal myopathy induced by monensin in adult turkeys. *Aust Vet J* 68:250–251.

Podell M, Valentine BA, Cummings JF, et al: 1995. Electromyography in acquired equine motor neuron disease. *Prog Vet Neurol* 6:128–134.

Free ebooks ==> www.Ebook777.com

Porciello F, Rishniw M, Herndon WE, et al: 2008. Cardiac troponin I is elevated in dogs and cats with azotaemia renal failure and in dogs with non-cardiac systemic disease. *Aust Vet J* 86:390–394.

Prosek R, Sisson D, Oyama M, et al: 2007. Distinguishing cardiac and non-cardiac dyspnea in 48 dogs via plasma atrial natriuretic factor, B-type natriuretic factor, endothelin and cardiac troponin I. *J Vet Intern Med* 21:238–242.

Radostits OM, Gay CC, Blood DC, Hinchcliff, KW: 1999. Diseases of the Musculoskeletal System. *In:* Radostits OM, Gay CC, Blood DC, Hinchcliff, KW (eds): *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 9th ed. Saunders, New York, pp. 551–578.

Ritchie BW: 1998. Interpreting the avian CBC and serum chemistry profile. *Proc North Am Vet Conf* 12:783–784.

Sharkey LC, Berzina I, Ferasin L, et al: 2009. Evaluation of serum cardiac troponin I concentration in dogs with renal failure. *J Am Vet Med Assoc* 234:767–770.

Shell LG: 1997. Diseases of Peripheral Nerve, Neuromuscular Junction, and Muscles, *In:* Leib MS, Monroe WE (eds): *Practical Small Animal Internal Medicine*. W.B. Saunders, Philadelphia, pp. 591–612.

Shelton GD: 2000. Myopathy. *In:* Tilley LP, Smith FWK (eds): *The 5-Minute Veterinary Consult: Canine and Feline*, 2nd ed. Lippincott Williams and Wilkins, Philadelphia, pp. 984–989, 994–995.

Shelton GD, Ho M, Kass PH: 2000. Risk factors for acquired myasthenia gravis in cats: 105 cases (1986–1998). *J Am Vet Med Assoc* 216:55–57.

Solter PF: 2007. Clinical Biomarkers of Cardiac Injury and Disease. *In: Proceedings of the ACVP/ASVCP Annual Meetings*, Savannah, Georgia.

Spratt DP, Mellanby RJ, Drury N, et al: 2005. Cardiac troponin I: Evaluation of a biomarker for diagnosis of heart disease in the dog. *J Small Anim Pract* 46:139–145.

Stockham SL, Scott MA: 2008. *Fundamentals of Veterinary Clinical Pathology*, 2nd ed. Blackwell, Ames, IA, pp. 639–674.

Swenson CL, Graves TK: 1997. Absence of liver specificity for canine alanine aminotransferase (ALT). *Vet Clin Pathol* 26:26–28.

Taylor SM: 1998. Disorders of Muscle. *In:* Nelson RW, Couto CG (eds): *Small Animal Internal Medicine*, 2nd ed. Mosby, St. Louis, pp. 1059–1075.

Thrall MA, Baker DC, Campbell TW, DeNicola D, Fettman MJ, Lassen ED, Rebar A, Weiser G: 2004. *Veterinary Hematology and Clinical Chemistry*. Lippincott Williams and Wilkins, Baltimore, pp. 417–420.

Valberg SJ, Hodgson DR, Carlson G, Parish, SM, Maas J: 2002. Diseases of Muscle. *In:* Smith BP *(ed): Large Animal Internal Medicine*, 3rd ed. Mosby, St. Louis, pp. 1266–1269, 1271–1272, 1274–1291.

Valberg S, Jonsson L, Lindholm A, et al: 1993. Muscle histopathology and plasma aspartate aminotransferase, creatine kinase and myoglobin changes with exercise in horses with recurrent exertional rhabdomyolysis. *Equine Vet J* 25:11–16.

Valentine BA, Blue JT, Shelley SM, et al: 1990. Increased serum alanine aminotransferase activity associated with muscle necrosis in the dog. *J Vet Intern Med* 4:140–143.

Valentine BA, Credille KM, Lavoie JP, et al: 1997. Severe polysaccharide storage myopathy in Belgian and Percheron draught horses. *Equine Vet J* 29:220–225.

www.Ebook777.com

van Kimmenade RRJ, Januzzi JL: 2009. The evolution of the natriuretic peptides—Current applications in human and animal medicine. *J Vet Cardiol 11*, Supplement 1:S9–S21.

Varga A, Schober KE, Holloman CH, et al: 2009. Correlation of serum cardiac troponin I and myocardial damage in cattle with monensin toxicosis. *J Vet Intern Med* 23:1108–1116.

Wilkins PA: 2002. White Muscle Disease. *In:* Brown C, Bertone J (eds): *The 5-Minute Veterinary Consult: Equine*, 1st ed. Lippincott Williams and Wilkins, Baltimore, pp. 1134–1135.

Free ebooks ==> www.Ebook777.com

CHAPTER 11

ENDOCRINE SYSTEM

Duncan C. Ferguson, VMD, PhD, and Margarethe Hoenig, Dr med vet, PhD

PARATHYROID GLAND, CALCIUM, PHOSPHORUS, AND MAGNESIUM

Parathyroid function is integrated with thyroid parafollicular cell function and vitamin D metabolism in the regulation of calcium and phosphorus homeostasis. Altered serum calcium and phosphorus concentrations are often encountered coincidentally during screening with biochemical profiles and in animals with clinical signs of altered calcium metabolism. Magnesium abnormalities are of greatest concern in ruminants due to feed intake, but also may be of concern in small animals with diabetic ketoacidosis. An overview of major disorders involving calcium, phosphorus, parathormone, and vitamin D metabolism is presented in Figure 11.1.

I. BASIC CONCEPTS

A. The ionized calcium-sensing receptor (CaSR) regulates the response of parathyroid chief cells, C-cells, and renal epithelial cells.

1. CaSR is present along the nephron on renal epithelial cells.

a. Stimulation of the CaSR by extracellular iCa concentration decreases NaCl, Ca, and Mg reabsorption in the proximal convoluted tubule and decreases water reabsorption in the collecting ducts.

b. Magnesium also is an agonist of the CaSR. Severe magnesium depletion decreases PTH secretion, increases resistance to PTH, and impairs calcitriol synthesis.

B. Parathormone (PTH) and parathormone-related peptide (PTHrp)

1. PTH is produced by the parathyroid gland in response to hypocalcemia and, to a lesser extent, in response to hypomagnesemia. Although serum phosphorus has no direct effect on PTH secretion, hyperphosphatemia may cause a reciprocal decrease in serum calcium and, thus, indirectly stimulate PTH release.

2. The net effect of PTH is increased serum calcium, decreased serum phosphorus, and increased renal excretion of phosphorus. PTH promotes the following:

a. Calcium release from bone

b. Phosphorus excretion by the kidney

c. Accelerated formation of the active form of vitamin D (1,25 dihydroxycholecalciferol) by stimulation of the 1-alpha-hydroxylase enzyme activity of the kidney

d. Calcium absorption from the gut

e. Calcium reabsorption by renal tubules

www.Ebook777.com

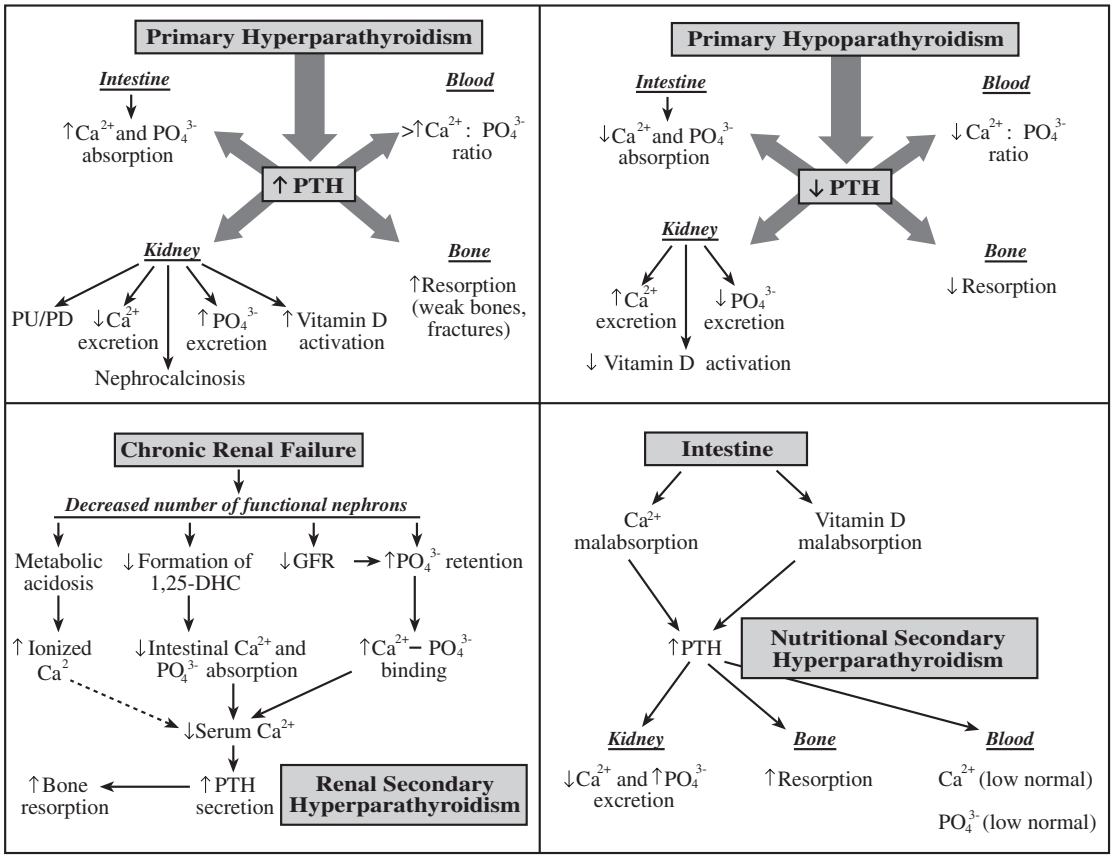


FIGURE 11.1. Overview of major disorders involving calcium, phosphorus, parathormone, and vitamin D metabolism.

A. Primary hyperparathyroidism. Increased synthesis of PTH by the parathyroid gland results in increased calcium and phosphorus absorption from the intestine, increased release of calcium and phosphorus from bone, increased renal retention of calcium, increased renal excretion of phosphate, and increased renal activation of vitamin D.

B. Primary hypoparathyroidism. Reduced PTH production by the parathyroid gland (because of agenesis, surgical removal, or atrophy) results in reduced calcium and phosphate absorption from the intestine, reduced release of calcium and phosphorus from bone, increased renal excretion of calcium, decreased renal excretion of phosphate, and reduced renal activation of vitamin D.

C. Renal secondary hyperparathyroidism. With a reduction in functional nephrons in chronic renal failure, metabolic acidosis develops. Metabolic acidosis increases the ionized calcium fraction. This effect is mitigated by reduced activation of vitamin D to 1,25-DHC by the kidney, which results in a decreased serum calcium concentration and increased retention of phosphate by the kidney. The serum calcium concentration may be further decreased via the calcium:phosphate solubility product. The reduction in serum calcium concentration leads to a secondary increase in PTH secretion, resulting in increased calcium and phosphate release from bone.

D. Nutritional or malabsorption secondary hyperparathyroidism. PTH concentration increases in response to decreased dietary intake of calcium and/or vitamin D or malabsorption that results in reduced availability of these nutrients. The increased PTH concentration promotes renal retention of calcium, renal excretion of phosphate, and bone resorption (resulting in rubberjaw). Serum calcium and phosphate concentrations generally are decreased but remain within the reference interval.

PTH = parathyroid hormone

Ca^{2+} = calcium

PO_4^{3-} = phosphate

1,25-DHC = 1,25 dihydroxycholecalciferol

GFR = glomerular filtration rate

PU/PD = polyuria and polydipsia

Free ebooks ==> www.Ebook777.com

3. PTHrp has virtually identical biological activities as PTH except in mammary tissue. The physiological role of PTHrp also may include stimulation of placental calcium transport in the newborn.

4. Quantitation of PTH and PTHrp

a. A radioimmunoassay is commercially available to quantitate intact PTH in the serum of dogs, cats, and horses.

b. A research radioimmunoassay is available for PTHrp produced by round cell malignancies (e.g., lymphoma).

C. Calcitonin (thyrocalcitonin)

1. Calcitonin is produced by thyroid parafollicular cells (C cells) in response to hypercalcemia.

2. Calcitonin's effects are to decrease serum calcium and phosphorus. It produces these changes through the following mechanisms:

a. Inhibition of PTH-stimulated bone resorption

b. Increased phosphorus excretion by the kidney

3. In concert with PTH, calcitonin regulates blood calcium concentration within precise limits and tempers the PTH resorptive action on bone.

4. Although a research assay has been developed for dogs, cattle, horses, and birds, validated assays for quantitation of calcitonin are not commercially available for animals.

D. Metabolically active vitamin D

1. 25-hydroxylation of vitamin D to 25-hydroxycholecalciferol (25-OH-D3) occurs in the liver. 1-alpha hydroxylation of 25-OH-D3 to 1,25-dihydroxycholecalciferol occurs under PTH regulation in the kidney.

2. 1,25-dihydroxycholecalciferol is the metabolically active form of vitamin D. It promotes calcium and phosphorus absorption by the intestinal mucosa and may facilitate PTH action on bone.

3. Immunoassays and HPLC procedures to quantitate 25-OH-D3 and 1,25-dihydroxycholecalciferol are available at specialty diagnostic laboratories. The major application of these assays is to confirm vitamin D toxicosis.

E. Calcium

1. Total serum calcium exists in three forms.

a. Ionized calcium (iCa) accounts for approximately 50% of total serum calcium, and is the most important biologically active fraction.

b. Protein-bound calcium represents approximately 40% of total serum calcium. Albumin is the major protein to which calcium is bound in serum. Protein-bound calcium (pCa) acts as a buffering system, diminishing acute changes in the iCa fraction.

c. Chelated or complexed calcium and magnesium is formed largely with phosphate, citrate, bicarbonate, sulfate, and lactate anions, and accounts for approximately 10% of total serum calcium.

2. Ionized calcium

a. Only ionized calcium is biologically active in bone formation, neuromuscular activity, cellular biochemical processes, and blood coagulation.

b. The proportion of total calcium that is ionized is affected by acid-base balance.

(1) Alkalosis decreases serum ionized calcium concentration.

(a) Metabolic alkalosis may cause subclinical hypocalcemia.

(b) Milk fever in cows is the result of an alkalizing diet supplying more cations (Na, K, Ca and Mg) than anions.

(c) Alkalosis blunts tissue responses to PTH.

(2) Acidosis increases serum ionized calcium concentration.

c. Ionized calcium is almost always increased in hypercalcemic conditions.

www.Ebook777.com

d. Ionized calcium is almost always decreased (less than 6.5 mg/dL or 1.3 mmol/L) in severe hypocalcemia.

3. The protein-bound fraction and total serum calcium decrease in hypoalbuminemia, but ionized calcium remains within the reference interval.

a. Although hypocalcemia is found in hypoalbuminemic conditions, clinical signs of hypocalcemia do not occur.

b. In hypoalbuminemic dogs, measured serum calcium can be adjusted with a correction formula to help exclude the possibility of functional hypocalcemia:

$$\text{adjusted calcium (mg/dL)} = 3.5 - \text{albumin (g/dL)} + \text{measured calcium (mg/dL)}.$$

c. An alternative formula uses total protein measurements:

$$\text{adjusted calcium (mg/dL)} = \text{measured calcium (mg/dL)} - (0.4 \times \text{serum protein [g/dL]}) + 3.3\ .$$

d. Research and clinical studies have demonstrated that ionized calcium does not always parallel the predictions of these formulae. Therefore, direct measurement of ionized calcium is preferred.

e. In dogs, serum total calcium overestimates normocalcemia and underestimates hypocalcemia.

f. Use of a calcium adjustment formula to correct tCa to total protein or albumin concentration overestimates hypercalcemia and underestimates hypocalcemia.

4. Dietary intake of calcium seldom affects the serum calcium concentration directly.

a. Reduced intake of calcium is accommodated by increased PTH activity, and normocalcemia occurs.

b. Increased calcium intake is balanced by exogenous fecal loss and urinary excretion.

5. Special comments about horses

a. Compared to other species, horses have been noted as having high serum total Ca and iCa, poorly regulated intestinal Ca absorption, high urinary fractional clearance of calcium, low serum concentrations of vitamin D metabolites, and an increased Ca setpoint.

b. Reduced renal excretion of calcium causes hypercalcemia in some horses with renal disease; however, renal failure in other species usually does not produce hypercalcemia (Chapter 9).

6. Methods of measurement

a. Colorimetric

(1) Colorimetric methods measure total serum calcium (i.e., ionized, protein-bound, and complexed forms of calcium).

(2) False-high calcium values may occur in some assay systems when lipemia is present.

b. Ion-selective electrodes

(1) Special ion-selective electrodes are required to measure ionized calcium, the biologically active form of this metal.

(2) Samples must be maintained anaerobically at 37°C, which limits clinical utility of this test method.

(3) The amount and type of heparin used for whole blood or plasma samples also may affect the measurement of iCa.

(a) Zinc heparin leads to overestimation of iCa due to decrease in pH, which displaces Ca from proteins.

(b) Lithium heparin leads to underestimation of iCa.

(c) Consistency in the blood/heparin volume ratios is important unless dry heparin is used.

c. Urinary calcium excretion

(1) Differentiation of diseases causing abnormal serum calcium concentrations can be facilitated by measuring urinary calcium excretion.

(2) A 24-hour urine collection is made, and the amount of calcium excreted/24 hours is determined. Difficulty of sample collection limits the practical value of this technique.

Free ebooks ==> www.Ebook777.com

(3) A simpler method is to determine the fractional clearance of calcium on samples of serum and urine collected at the same time.

$$FE = (\text{urine } Ca^{++}/\text{serum } Ca^{++}) \times (\text{serum creatinine}/\text{urine creatinine}) .$$

See Chapter 9 for additional discussion on fractional clearance of electrolytes.

F. Phosphorus

1. Phosphorus, in its various anionic forms, functions with phosphoric acid as a buffer system in body fluids. However, acid-base balance is evaluated by measuring components of the bicarbonate buffer system instead of the phosphate buffer system because the latter is predominately an intercellular ion (Chapter 5).

2. Serum phosphorus is regulated primarily by the kidneys. When the tubular reabsorption maximum is exceeded, phosphaturia occurs.

a. PTH may enhance phosphaturia by decreasing tubular reabsorption of phosphorus.

b. Dietary intake of phosphorus may directly affect the serum phosphorus concentration.

c. Abnormal serum phosphorus concentration is caused by altered dietary concentrations, decreased renal excretion, and hormonal imbalances that affect serum calcium concentration.

3. Methods of measurement

a. Colorimetric methods measure inorganic phosphate that is present in bodily fluids as HPO_3^- and H_3PO_4 (primarily the former).

b. Hemolyzed serum may cause falsely high or falsely low phosphorus values, depending upon the specific assay.

c. Young animals have higher serum phosphorus concentrations than adults due to active skeletal growth.

d. Urinary excretion over 24 hours or fractional renal clearance determination may help evaluate phosphorus metabolism. Fractional clearance of phosphorus is determined in the same manner as fractional clearance of calcium.

G. Magnesium

1. Ionized magnesium is the biologically active fraction, and is important in support of enzyme activity, being a cofactor for many enzymes. Biologic roles for protein-bound magnesium and complexed magnesium have not been investigated.

2. Serum magnesium concentration depends on dietary intake and is regulated by mineralocorticoids and PTH.

3. Colorimetric laboratory methods are used to measure serum and urine magnesium concentrations.

4. Abnormal magnesium metabolism and associated diseases are described primarily in ruminants. However, magnesium and phosphate depletion has been documented in insulin-treated dogs with diabetic ketoacidosis.

5. Magnesium concentration occasionally is monitored in small animals on total parenteral nutrition.

II. LABORATORY ABNORMALITIES IN CALCIUM, PHOSPHORUS, AND MAGNESIUM IMBALANCE

A. Hypercalcemia

1. Based upon one large study, hypercalcemia was relatively common and occurred in 19% of dogs and 17% of cats.

2. Primary hyperparathyroidism is caused by functional parathyroid neoplasms or idiopathic hyperplasia (Figure 11.1A).

www.Ebook777.com

a. Serum PTH concentration is high or, occasionally, within the reference interval in affected dogs. A PTH value that is within the reference interval is consistent with hyperparathyroidism in a hypercalcemic, hypophosphatemic dog that is not azotemic.
b. Increased urinary excretion of calcium and decreased urinary excretion of phosphorus in hyperparathyroid dogs seem to conflict with the known effects of PTH. However, hypercalcemia and hypophosphatemia, which are usually marked in these dogs, explain the urinary findings.
c. Hypophosphatemia is severe, but serum phosphorus may increase as nephrocalcinosis and renal failure develop.
d. Bone lesions, soft-tissue mineralization, and increased serum alkaline phosphatase activity may occur.
e. Isosthenuria is common in affected dogs.

3. Pseudohyperparathyroidism associated with neoplasia
a. Neoplasia is the most common cause of symptomatic hypercalcemia in dogs and also has been reported as a cause of hypercalcemia in cats and horses.
b. The mechanisms that produce hypercalcemia in affected animals are multiple and vary with the type of tumor.
(1) A variety of neoplasms may produce PTHrp, a vitamin-D-like steroid, a prostaglandin, or osteoclast-activating factor, any of which may cause hypercalcemia and hypophosphatemia.
c. Lymphoma has been associated with hypercalcemia in several animal species and is the most common cause of tumor-associated hypercalcemia. More than 90% of dogs with lymphoma and hypercalcemia have enlarged lymph nodes (Case 10).
d. Apocrine gland adenocarcinoma of the anal sac is the second most commonly associated tumor in dogs with PTHrp-associated hypercalcemia. More than 50% of affected dogs are hypercalcemic at the time of tumor diagnosis.
e. Hypercalcemia in multiple myeloma is partially the result of an osteoclast-activating factor secreted by neoplastic plasma cells.
f. Hypercalcemia occasionally occurs in animals with other neoplasms, primarily carcinomas (e.g., squamous cell carcinoma, nasal carcinoma, ovarian stromal tumor, thymoma). Secretion of PTH-related protein (PTHrp) by some carcinomas results in hypercalcemia.
g. Hypophosphatemia may be severe, but serum phosphorus concentration may increase after the onset of nephrocalcinosis and renal failure.
h. Serum PTH concentration is decreased.

4. Other causes of hypercalcemia
a. Hypervitaminosis D is an uncommon cause of hypercalcemia, but occurs occasionally after excessive dietary supplementation.
b. Ingestion of cholecalciferol-containing rodenticides produces acute vitamin D toxicosis.
c. Ingestion of plants that contain vitamin D glycosides (e.g., *Cestrum* sp. and *Solanum* sp.) may cause hypercalcemia, parathyroid atrophy, and soft tissue mineralization.
d. Renal disease in horses often is associated with hypercalcemia.
(1) Decreased renal calcium excretion may cause nephrocalcinosis and mineralization of other soft tissues.
(2) The equine kidney excretes large amounts of calcium in health.
(3) The likelihood of hypercalcemia occurring in a given horse with renal disease may be related to the calcium content of the diet.
e. Renal failure in dogs infrequently may be accompanied by hypercalcemia.
(1) Hypercalcemia occurs most frequently in relatively young dogs with familial renal disease (e.g., Lhasa Apso).
(2) Hypercalcemia is not a common laboratory abnormality in aged dogs with chronic renal failure.
f. Canine adrenal insufficiency often is accompanied by hypercalcemia.

Free ebooks ==> www.Ebook777.com

(1) The mechanism, which appears to be associated with glucocorticoid deficiency, involves reduced calcium excretion in the urine and possibly reduced antagonism of vitamin D on intestinal absorption of calcium.
(2) Additional laboratory abnormalities include hyponatremia, hyperkalemia, hyperphosphatemia, and azotemia.
(3) Ionized calcium is normal in affected dogs.

g. Bone disease that is accompanied by osteolysis (e.g., septic osteomyelitis, metastatic neoplasia) may produce hypercalcemia.

h. Granulomatous disease, especially blastomycosis, occasionally is accompanied by hypercalcemia in dogs. Excessive production of 1,25 dihydroxycholecalciferol by macrophages has been suggested as the causative mechanism.

4. The severity of hypercalcemia may be masked when concomitant hypoalbuminemia is present, provided that total serum calcium is being measured.

B. Hypocalcemia (Table 11.1).

1. Relatively common as follows:

a. Based on serum iCa (ionized calcium), one study showed the prevalence of hypocalcemia to be 31% in sick dogs and 27% in sick cats.

b. In a critical care population of horses with enterocolitis, iCa was low in nearly 80% of the cases. Of these cases, 71% had elevated PTH and 29% was inappropriately normal or low.

c. Foals with sepsis also have a high incidence of hypocalcemia

d. Recent research has shown that a systemic inflammatory response leads overexpression of CaSR in equine parathyroid cells, resulting in a blunted PTH response to hypocalcemia.

2. Hypoalbuminemia (in all species) and alkalosis (especially in ruminants) are common causes of hypocalcemia that must be excluded in each patient (Cases 13, 14, 16, 18, 19).

3. Other causes of hypocalcemia include chronic renal failure, critical illness, hemorrhage, gastrointestinal disease, pancreatitis, diabetes mellitus, lactation, urethral obstruction, renal transplantation, vitamin D deficiency, and hypoparathyroidism.

TABLE 11.1
CAUSES OF HYPERCALCEMIA AND HYPOCALCEMIA.

Hypercalcemia	Hypocalcemia
Acidosis	Acute pancreatitis
Granulomatous disease (blastomycosis)	Alkalosis
Hemoconcentration	C-cell thyroid neoplasms
Hyperproteinemia, including paraproteinemia	Eclampsia, milk fever
Hypoadrenocorticism	EDTA
Hyperparathyroidism	Enterocolitis (adult horses)
Immobilization	Hypomagnesemic tetany (grass tetany)
Neoplasia	Hypoparathyroidism
Apocrine gland tumors of the anal sacs	Hypoproteinemia (hypoalbuminemia)
Carcinomas metastatic to bone	Malabsorption
Lymphoma	Phosphate enema, phosphate-containing fluids
Plasma cell myeloma	Renal failure
Osteolytic lesions	Sepsis (foals)
Renal failure	Toxicosis
Toxicosis	Blister beetle (cantharidin) toxicosis
Calciferol-containing rodenticides	Ethylene glycol toxicity
Hypervitaminosis D	
Vitamin D glycoside-containing plants	

www.Ebook777.com

4. Either acute or chronic renal disease (CRD) in dogs, cats, and ruminants may cause hypocalcemia through several mechanisms, including decreased formation of 1,25-dihydroxycholecalciferol and soft tissue deposition of calcium salts secondary to hyperphosphatemia (Cases 15, 22).
   a. In one large case series, hypocalcemia was observed in 36% of 490 dogs with CRD and 10% of 102 cats with CRD. In cats with CRD, hypocalcemia is found more frequently with more advanced magnitudesof azotemia
   b. Acute renal failure in ethylene glycol toxicosis may be accompanied by profound hypocalcemia because of the chelation of calcium by oxalate (Case 18).
   c. Postrenal urinary obstruction in cats may cause hypocalcemia and hyperphosphatemia.
   d. Uremic acidosis may temper the severity of hypocalcemia because the proportion of ionized calcium in serum is increased (Case 20).
5. Hypoparathyroidism is described in dogs, cats, and horses. It may occur spontaneously or following surgical removal of thyroid and parathyroid tissue (as in surgically treated hyperthyroid cats). The latter accounts for most cases of hypoparathyroidism; however, this surgical complication has become less common with newer surgical techniques designed to preserve parathyroid tissue.
   a. As with hypercalcemia, PTH concentrations should be interpreted in conjunction with serum calcium or, preferably, serum ionized calcium concentrations. Serum iCa is low and PTH concentration is decreased or within the reference interval in animals with primary hypoparathyroidism.
   b. Serum phosphorus concentration is within the reference interval or mild hyperphosphatemia is present.
   c. Patients with low iCa and increased PTH have parathyroid-independent hypocalcemia.
6. Approximately 50% of dogs with acute pancreatitis have hypocalcemia.
   a. Multiple mechanisms have been suggested as the cause of hypocalcemia, including the following:
      (1) Formation of calcium 'soaps' in the pancreas
      (2) Hypomagnesemia
      (3) Decreased PTH secretion
      (4) Hypoproteinemia
      (5) Glucagon-stimulated calcitonin secretion
      (6) Amylin, which is normally secreted from the endocrine pancreas along with insulin, is structurally related to calcitonin and has weak calcitonin-like activity on calcium metabolism.
   b. No single factor has been proven to be the cause of hypocalcemia in pancreatitis (Case 14).
   c. In most cases of pancreatitis, coexisting acidosis results in an increase in the ionized fraction of calcium and limits the possibility of tetany.
7. Parturient paresis (milk fever) in cattle is caused by hypocalcemia. Puerpural tetany (eclampsia) in the bitch, mare, or ewe is similar.
   a. In the cow, parturient paresis accompanies the sudden demand for calcium during lactation.
   b. An increased incidence of parturient paresis may occur after high-calcium diets are fed in the dry period. Such diets depress the ability of the animal to respond to the intense calcium demand of lactation.
   c. Other concurrent metabolic abnormalities probably contribute to the paresis.
   d. Affected cows usually have concurrent hypophosphatemia.
8. Approximately 75% of cows with hypomagnesemic tetany (grass tetany) also have concurrent hypocalcemia.
9. Intestinal malabsorption may produce hypocalcemia via decreased absorption of calcium and vitamin D (Case 16).
10. Other uncommon causes of hypocalcemia include phosphate enemas in cats, blister beetle toxicosis in horses, and hypercalcitonism in bulls with thyroid C-cell neoplasms.

Free ebooks ==> www.Ebook777.com

TABLE 11.2
CAUSES OF HYPERPHOSPHATEMIA AND HYPOPHOSPHATEMIA.

Hyperphosphatemia
- Hemolysis (*in vitro*)
- Hypervitaminosis D
- Hypoparathyroidism
- Nutritional secondary hyperparathyroidism
- Osteolytic bone lesions
- Phosphate enema, phosphate-containing fluids
- Renal failure
- Tumor lysis syndrome
- Young animals

Hypophosphatemia
- C-cell thyroid neoplasms
- Hemolysis (*in vitro*)
- Hyperinsulinism, insulin therapy
- Hyperparathyroidism
- Milk fever
- Neoplasia

Renal failure (horse)

C. Hyperphosphatemia (Table 11.2)

1. The most common mechanism of hyperphosphatemia is decreased glomerular filtration rate associated with various causes of prerenal, renal, and postrenal azotemia (Cases 15, 17, 18, 19, 20, 34; Chapter 9).

2. Hyperphosphatemia that is secondary to disturbances of calcium metabolism has been described above, under disorders of hypercalcemia and hypocalcemia.

a. Hyperphosphatemia that accompanies hypercalcemia and precedes renal failure may occur with hypervitaminosis D.

b. Hyperphosphatemia that accompanies hypocalcemia occurs with hypoparathyroidism and nutritional secondary hyperparathyroidism from excessive dietary phosphorus.

3. Hyperphosphatemia may result from increased intestinal absorption of phosphorus related to high-phosphorus diets or phosphate-containing enemas.

4. Osteolytic bone lesions may cause hyperphosphatemia.

D. Hypophosphatemia (Table 11.2)

1. Hypophosphatemia is an uncommon laboratory abnormality, the cause of which is often undetermined.

2. Primary hyperparathyroidism and pseudohyperparathyroidism cause hypophosphatemia (see description under hypercalcemia, above).

3. Nutritional secondary hyperparathyroidism, caused by dietary lack of calcium or hypovitaminosis D, is associated with hypophosphatemia or a serum phosphorus value that is within the reference interval.

4. Other causes of hypophosphatemia include the following:

a. Inadequate dietary phosphorus intake or intestinal malabsorption

b. Milk fever and eclampsia

www.Ebook777.com

c. Hyperinsulinism or insulin administration
d. Hyperalimentation
e. *In vitro* hemolysis with some laboratory analytical methods

E. Hypermagnesemia
1. Hypermagnesemia is an uncommon laboratory abnormality because the kidneys readily excrete excessive magnesium.
2. Hypermagnesemia may occur in herbivores with renal failure.
3. Administration of antacids or magnesium cathartics to patients with renal failure may result in hypermagnesemia.

F. Hypomagnesemia
1. Hypomagnesemia has been associated with hypoparathyroidism, critical illness, diabetes mellitus, lactation tetany, and protein-losing enteropathy.
2. Hypomagnesemia is of greatest concern in calves, adult cattle, and sheep.
a. Both slow-onset and acute-onset clinical forms of hypomagnesemia are described.
b. Concurrent hypocalcemia may be refractory to treatment until hypomagnesemia is corrected.
3. Inadequate dietary magnesium intake is related to the development of clinical disease (i.e., grass tetany), but the pathogenesis of this disease is poorly understood.
4. Grass tetany is the classical presentation of hypomagnesemia in cattle, but asymptomatic hypomagnesemia also occurs frequently in adult cattle.
5. Urine magnesium concentration can be useful in diagnosis of syndromes associated with hypomagnesemia.
a. Healthy cattle have urine magnesium concentrations of approximately 50 mg/dL.
b. In hypomagnesemic states, the renal tubular absorptive mechanism may capture almost all magnesium in the glomerular filtrate, resulting in urine magnesium concentrations of less than 5 mg/dL.

THYROID FUNCTION

The principal thyroid hormones, thyroxine (T4) and triiodothyronine (T3), increase metabolism of most cells and stimulate growth in the young. They induce DNA translation, which results in the production of proteins associated with cell growth, oxidative phosphorylation, and membrane transport of electrolytes. The hypothalamic-pituitary-thyroid axis is presented in Figure 11.2.

I. BASIC CONCEPTS

A. Secretion and transport of T4 and T3
1. In the thyroid glands of dogs and cats, approximately 80% of the secreted thyroid hormone is T4 and 20% is T3.
2. T3 is approximately four-fold more potent than T4, but both hormones function at the cellular level.
a. 50% of serum T3 in dogs and 80% in cats is derived from deiodination of T4 outside of the thyroid gland by 5'-deiodinase enzymes.
(1) Type I 5'-deiodinase (D1)
(*a*) Present in tissues such as liver and kidney.
(*b*) T3 produced by D1 contributes significantly to circulatory T3 concentration for distribution to other tissues.
(*c*) Activity of D1 is reduced in hypothyroidism and increased in hyperthyroidism.
(*d*) D1 activity is reduced by protein-calorie malnutrition and severe illness.

Free ebooks ==> www.Ebook777.com

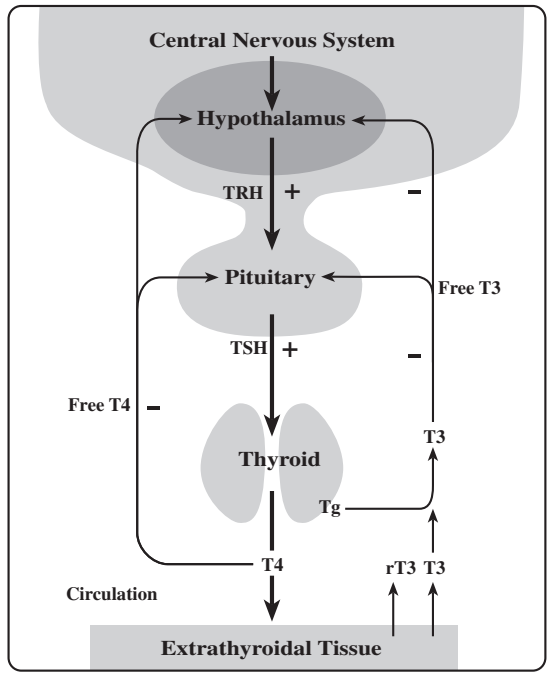


FIGURE 11.2. Overview of the hypothalamic-pituitary-thyroid (HPT) axis in euthyroid animals. The hypothalamus produces thyrotropin releasing hormone (TRH) that is released into the pituitary portal system. TRH stimulates the anterior pituitary thyrotrope cells to produce thyrotropin (thyroid stimulating hormone, TSH). TSH promotes the growth and secretory function of the thyroid glands. The thyroid glands produce both thyroxine (T4) and 3,5,3'-triiodothyronine (T3) and release these hormones into the systemic circulation. Approximately 50% of the T3 is produced by the thyroid gland and 50% is produced by extrathyroidal tissue conversion of T4 to T3 by 5'-deiodinase enzymes. In the circulation, thyroid hormones are highly bound by serum-binding proteins. Free T4 (FT4) is monitored by the hypothalamus and pituitary, serving as a negative feedback for the regulation of TRH and TSH secretion.

(e) D1 activity is inhibited by drugs such as propylthiouracil and iodinated radiocontrast agents such as iopanoic acid.

(2) Type II 5'-deiodinase (D2)

(a) Present in the brain, pituitary, and central nervous system of most species, and has been identified in the skin of dogs.

(b) T3 produced by D2 largely is retained at the tissue site and does not contribute to serum T3 concentration under euthyroid conditions but probably maintains serum T3 concentrations during hypothyroidism.

(c) D2 activity is increased in hypothyroidism and decreased in hyperthyroidism.

(d) Serum TSH (or its logarithm) is inversely proportional to circulating free T4 concentrations:

i) Virtually all of the available (free) T4 fraction entering the pituitary is converted to T3 by type II 5'-deiodinase.

ii) A negative feedback effect that reduces pituitary TSH release is triggered when generated T3 interacts with pituitary nuclear receptors.

b. T4 also may be deiodinated to reverse T3 (rT3), a metabolically inactive molecule, and T3 is inactivated by the 5-deiodinase enzyme (type III deiodinase, D3).

(1) D3 expression is high in some tissues under development (e.g., nervous system) and white blood cells at sites of inflammation.

www.Ebook777.com

(2) When D1 is inhibited, the clearance of rT3 is also decreased, resulting in increased rT3 concentration.

(3) Under most circumstances, rT3 concentration generally parallels that of unbound (free) T4.

c. In impending thyroid failure (primary hypothyroidism) and in iodine deficiency, serum T3 tends to be maintained within the reference interval longer than T4.

(1) Maintenance of T3 concentration presumably is due to both increased thyroidal and peripheral 5'-deiodination from the diminished amount of T4 substrate being made by the thyroid gland.

(2) For the above reasons, serum T3 concentration generally does not decline in early or mild primary thyroid failure.

d. Thyroid hormone secretion is regulated by the pituitary gland through thyroid-stimulating hormone (TSH), which stimulates thyroid secretion.

(1) Thyrotropin-releasing hormone (TRH), a hypothalamic hormone, is essential for normal TSH secretion by the pituitary.

(2) Negative feedback control of TSH secretion is mediated by the unbound fractions of T3 and T4 at both the hypothalamic and pituitary levels.

3. Significant species differences in binding protein quantities exist.

a. Approximately 99.9% of circulating T4 and 99% of circulating T3 are bound to plasma proteins in most non-primate mammalian species.

b. In the dog, T4 and T3 are bound to thyroid-binding globulin (TBG) as well as thyroxine-binding pre-albumin (TBPA), albumin, and lipoproteins.

(1) Serum TBG concentrations are low in dogs (15%) and undetectable in cats compared to those in man.

(2) Decreased TBG concentrations in dogs and cats largely account for lower serum T4 concentrations and higher free T4 fractions in these species.

(3) In reptiles and birds, total T4 concentrations are extremely low, due in part to the absence of some high-affinity serum-binding proteins.

c. Free T4 (fT4) and free T3 (fT3) are the fractions of these hormones which are not protein bound. Thus, circulating fT4 and fT3 represent the fraction available to tissues at steady-state and are generally proportionate to the true thyroid status.

(1) The free T4 fraction (fT4/total T4 expressed as a percentage) averages 0.1% in healthy dogs, cats, and horses.

(2) Free T4 concentrations, measured by dialysis methods, are fairly consistent between species. While reference intervals should be determined for the laboratory performing the test, fT4 often averages approximately 2 ng/dL or 25 pmol/L.

B. Measurement of serum T4

1. Commercial immunoassays are used to quantitate T4 in canine, feline, and equine serum samples.

a. Most of these commercial assay kits are designed to measure T4 in human sera, in which higher concentrations of T4 are encountered in health.

b. Commercial assay kits for use with human sera are inaccurate at the low T4 concentrations often found in animal sera.

c. Human commercial assay kits may be modified for use with animal serum samples by preparing standards with lower T4 concentrations.

(1) These standards are prepared in thyroid-hormone-depleted serum from the species of interest.

(2) The concentration of the anti-T4 antibody reagent often must be diluted to obtain optimal test sensitivity.

Free ebooks ==> www.Ebook777.com

2. Total thyroxine (T4) radioimmunoassays measure both bound and unbound (free, fT4) hormone.
3. Alterations in serum T4 concentration can occur with nonthyroidal illness and drug administration.
   a. Hypoproteinemia may result in decreased total T4 concentration, even though the animal is euthyroid. Free T4 concentrations are then within the reference interval.
   b. Total T4 concentration gradually declines with age in dogs and horses but not in cats.
   c. Increased serum total T4 concentration in dogs and increased serum fT4 concentration in cats may be observed with obesity, but usually remains within the normal range and does not reflect a condition of hyperthyroidism.
   d. Severe illness results in decreased total serum T4 concentrations in dogs and cats; a poorer clinical prognosis is associated with a lower total T4 concentration.
   e. Small breeds of dogs tend to have slightly higher reference intervals for serum T4 concentration. Therefore, reference intervals for individual breeds should be established, if practical.
   f. Administration of glucocorticoids, sulfonamides, phenobarbital, and nonsteroidal analgesics may reduce total serum T4 concentration. Higher seizure frequency, like other nonthyroidal illnesses, has been shown to depress T4 concentrations.
   g. Decreased serum-free T4 concentration and increased TSH also has been associated with sulfonamide administration, indicating a drug-induced form of hypothyroidism.
   h. In a recent study, serum T4 concentration decreased significantly from baseline during surgery and remained low at one, two, four, and 24 hours postoperatively. Serum T3 was depressed at one hour and serum rT3 was increased at eight, 12, 24, and 36 hours. Serum fT4 increased significantly from baseline in the surgery group compared to the control at one and seven days and compared to the anesthesia-only group at two days.
4. Interpretation of total T4 concentration
   a. Serum total T4 concentration is within the reference interval.
      (1) Hypothyroidism generally can be excluded.
      (2) In 10% of cases of hypothyroidism, the presence of anti-T4 autoantibodies may increase total T4 concentration into (9%) or above (1%) the reference interval and hypothyroidism will not be detected.
   b. Serum total T4 concentration is decreased (Cases 31 and 32).
      (1) Hypothyroidism may be present (see below).
      (2) A decreased serum total T4 concentration does not confirm hypothyroidism, even when this disease is suspected clinically.
      (3) Nonthyroidal illness may depress the serum total T4 concentration.
      (4) If the serum total T4 concentration is decreased and nonthyroidal illness is present based upon medical history, physical examination, and routine laboratory testing (i.e., complete blood cell [CBC] count and biochemical profile), hypothyroidism should be confirmed or excluded by determining TSH and/or fT4 concentrations (see below).
   c. Serum total T4 concentration is increased.
      (1) Hyperthyroidism may be present.
      (2) If the serum total T4 concentration is increased when hypothyroidism is suspected (approximately 1% of hypothyroid individuals), the patient's serum should be tested for anti-T4 autoantibodies.

C. Measurement of serum T3
1. Immunoassays for quantitation of serum T3 concentration are available.
   a. Serum T3 concentration is lower than serum T4 concentration.
   b. A larger volume of serum is needed to measure T3 concentration.

www.Ebook777.com

c. In constructing the standard curve, it is critical to include hormone-free serum from the species being tested.

2. T3 is the most active form of thyroid hormone, but serum T3 concentrations correlate poorly with clinical disease largely because the body has both thyroidal and peripheral tissue mechanisms to increase the T3/T4 ratio in early thyroid failure.

3. Little diagnostic value is gained by routinely quantitating serum T3 concentration.

4. In some animals with hypothyroidism and thyroiditis, falsely high or falsely low T3 concentrations can result from the presence of anti-T3 autoantibodies in the serum (see below).

a. High T3 concentrations are obtained with assays, which capture the antibody form of the hormone.

b. Low or undetectable T3 concentrations are produced by assays, which depend upon quantifying the antibody unbound radioactive hormone by charcoal or other extractants.

D. Measurement of serum-free T4 (fT4)

1. Analytical methods

a. A commercial two-step analytical method using equilibrium dialysis is the only widely validated procedure for determination of fT4 in domestic animals.

b. All commercial non-dialysis fT4 immunoassays have been prepared with reagents for human serum, which has more TBG than sera from most animal species.

c. Some laboratories' new two-step free T4 immunoassays may show better correlation with fT4 by equilibrium dialysis than older non-dialysis fT4 immunoassays.

d. Potential artifacts of most fT4 immunoassays

(1) The effects of low affinity inhibitors (e.g., fatty acids, highly protein bound drugs) are underestimated.

(2) Dilution may result in an underestimation of fT4 and potential overdiagnosis of hypothyroidism in the dog.

(3) fT4 concentration usually is increased in hyperthyroid cats.

(4) False-positive fT4 test results occur in approximately 5% of euthyroid cats.

(5) fT4 usually should be interpreted in conjunction with other thyroid tests.

(6) Despite potential limitations, the commercial, direct dialysis fT4 assay has the highest single-test diagnostic sensitivity, specificity, and accuracy in detecting thyroid disease.

e. Commercial one-step analytical methods

(1) Most of these assays are designed for human sera.

(2) These assays use solid phase antigen-linked tubes (SPALT) in which a thyroid hormone analogue linked to the assay tube competes for serum-binding proteins to quantitate the fT4 fraction.

(3) One-step analytical methods and two-step dialysis methods only correlate when sera from healthy animals are tested.

(4) Adequate correlation of fT4 by this method compared to the equilibrium dialysis method does not occur in the presence of serum-binding inhibitors due to illness or drug administration.

2. Effect of drug administration on fT4 (Case 33)

a. Heparin interferes with fT4 determination in vitro because it promotes the release of free fatty acids.

b. High concentrations of nonsteroidal anti-inflammatory drugs (NSAIDs) may displace T4. These drugs may include the following:

(1) Phenylbutazone in the horse

(2) Carprofen and aspirin in the dog

c. Furosemide displaces T4 in dog serum.

E. Influence of breed on T4 and fT4 concentrations in dogs

Free ebooks ==> www.Ebook777.com

1. Basal serum T4 and fT4 concentrations are decreased significantly in sighthounds (i.e., Greyhounds, Scottish Deerhounds, etc.) and Basenjis compared with other breeds.
2. Serum T4 concentrations after TSH and TRH administration are significantly lower in Greyhounds than in other breeds of dogs.
3. In sighthounds, serum T3 concentrations are more similar to those of non-sighthounds than either T4 or fT4 concentrations.
4. Ideally, breed-specific reference intervals should be established.

F. Measurement of endogenous thyrotropin (TSH) concentration (Cases 31, 32, 33)

1. TSH concentration is quantitated to detect a lack of negative feedback on the pituitary and hypothalamus.
2. TSH assays are available commercially for the dog but not for other animal species.
   a. Although a TSH assay is not commercially available for cats, about 35% cross-reactivity of the available canine TSH assay exists.
      (1) The canine TSH assay can detect elevated TSH, such as is seen following overtreatment of hyperthyroid cats with antithyroid drugs.
      (2) Generally, TSH is nondetectable in hyperthyroid cats. However, unlike in human medicine, the assay is not sensitive enough and undetectable TSH concentrations are seen in a significant number of euthyroid cats.
   b. A research TSH assay has been evaluated in horses.
3. If determination of serum TSH concentration is contemplated, it should be performed in conjunction with total T4 or fT4 quantitation.
4. Serum TSH concentrations are elevated in approximately 75% of dogs with primary hypothyroidism.
   a. A recent study has demonstrated that in chronically hypothyroid dogs (one year or longer), serum TSH may eventually fall into the normal range. The mechanism for this unique change in the dog has not yet been clarified, but may be due to either exhaustion of pituitary TSH secretion or a resetting of the pituitary-thyroid setpoint.
   b. Other theories for poor sensitivity of TSH as a screening assay for hypothyroidism.
      (1) Hypothyroid dogs may a have pulsatile release of TSH, possibly accounting for TSH values that are within the reference interval at certain times of the day.
      (2) The available TSH immunoassay system may not detect all glycosylation variants of circulating TSH in the dog.
5. Mean serum TSH concentrations do not appear to vary with breed.

G. Anti-thyroglobulin autoantibodies (Case 31)

1. Enzyme-linked immunosorbent assays (ELISA) are used to quantitate anti-thyroglobulin autoantibodies in dogs.
2. The presence of anti-thyroglobulin autoantibodies is indicative of thyroid autoimmunity (autoimmune thyroiditis).
3. Newer generation ELISA tests have fewer false-positive test results. False-positives in early versions of the assay have been reduced by routinely testing for non-specific binding.
4. Anti-thyroglobulin autoantibody ELISA tests appear to be highly specific for the presence of thyroid disease.
   a. Anti-thyroglobulin autoantibodies are present in 36% to 60% of dogs with hypothyroidism and in 91% of dogs with thyroiditis.
   b. False-positive test results occur at a rate of 5% to 6%.
   c. Anti-thyroglobulin autoantibody concentrations may be elevated prior to the onset of clinical signs of disease.
      (1) Approximately 20% of dogs with a positive anti-thyroglobulin autoantibody test exhibit one other test abnormality suggestive of hypothyroidism within one year.

www.Ebook777.com

d. Anti-thyroglobulin autoantibody, TSH, and fT4 testing have been suggested by the Thyroid Registry (Orthopedic Foundation for Animals) as screening tests for thyroid disease in breeding dogs; however, the results of these tests have not been demonstrated conclusively to have predictive value in the diagnosis of disease.

H. Anti-T4 and anti-T3 autoantibodies (Case 31)

1. Anti-T4 (approximately 15% of hypothyroid dogs) and anti-T3 (34% of hypothyroid dogs) autoantibodies occur in dogs with thyroiditis.

2. Anti-T4 and anti-T3 autoantibodies are measured in some diagnostic laboratories simply by diluting serum specimen in immunoassay buffer containing radioactive hormone. The percentage of hormone binding relative to an antibody-negative control is measured.

3. Sera that are positive for anti-T4 and anti-T3 autoantibodies almost always have a positive titer for anti-thyroglobulin (Tg) antibodies because the high-molecular-weight glycoprotein Tg is the likely immunogen and T3 and T4 antibodies may develop because they are haptens linked to Tg.

4. Clinical significance

a. Anti-T4 and anti-T3 autoantibodies have been observed in autoimmune thyroiditis; however, these antibodies also may be detected in some clinically healthy animals.

b. Anti-T4 and anti-T3 autoantibodies may interfere with accurate measurement of T3 or T4 by altering the amount of hormone-specific antibody in the tube relative to the standard curve, leading to very high or very low T3 or T4 concentrations.

(1) Very low T3 concentration has been misdiagnosed as poor conversion of T4 to T3, a condition which has not been identified outside of the pathophysiological conditions already described.

(2) Very high T3 and/or T4 concentrations in patients with clinical signs of hypothyroidism have been diagnosed inappropriately as thyroid hormone resistance.

(3) In the presence of anti-T4 immunoglobulin in a dog's serum, only fT4 concentration determined by the dialysis technique is accurate because the immunoglobulin does not pass into the dialysate prior to immunoassay.

(4) Accurate total T4 or total T3 concentrations can be determined only by research techniques such as ethanolic extraction of the hormone or heat inactivation of the immunoglobulin followed by chromatographic separation of the immunoglobulin fraction.

I. Comparison of single and multiple analytes for sensitivity, specificity, and accuracy in the diagnosis of primary hypothyroidism in dogs is presented in Table 11.3.

J. T4 response to TRH or TSH

1. T4 response to TRH or TSH administration is a test of thyroid reserve.

2. TSH stimulation test

a. The TSH stimulation test previously was performed with bovine TSH, which is no longer available **in a pharmaceutical preparation**.

b. Recombinant human TSH (rhTSH) administration is effective in animals but is very expensive.

(1) rhTSH at a fixed dose of 75 µg (approximately one unit) has beenrecommended and can be given repeatedly IV or IM to dogs with little adverse reaction.

(2) A recent study utilized 25 µg rhTSH in healthy cats, cats with NTI, and cats with low total T4 and azotemia following ^{131}I treatment. Total T4 and thyroidal pertechnetate were measured. Post-rhTSH serum T4 concentrations did not rise in the post-radioiodine group, whereas T4 concentrations in healthy and nonthyroidal illness groups increased significantly in response to TSH. The authors concluded that rhTSH stimulation allowed the differentiation of euthyroidism from iatrogenic hypothyroidism.

(3) Post TSH blood samples are generally drawn at four or six hours post-TSH given IV.

c. The TSH stimulation test is primarily a research technique but is considered to be the gold standard to diagnose thyroid disease.

Free ebooks ==> www.Ebook777.com

TABLE 11.3
COMPARISON OF SINGLE AND MULTIPLE ANALYTES FOR SENSITIVITY, SPECIFICITY, AND ACCURACY IN THE DIAGNOSIS OF PRIMARY HYPOTHYROIDISM IN DOGS.

	Percentage of Cases with Abnormal Test Result					
Test	**TT4**	**TT3**	**FT4D**	**TSH**	**TT4/TSH**	**FT4D/TSH**
Sensitivity	89/100*	10	98/80	76/86.7	67/86.7	74/80
Specificity	82/75.3	92	93/93.5	93/81.8	98/92.2	98/97.4
Accuracy	85	55	95	84	82	86

TT4 = total T4
TT3 = total T3
FT4D = free T4 determined by dialysis technique
TSH = thyroid stimulating hormone
TT4/TSH = total T4 to TSH ratio
FT4D/TSH = free T4 (determined by dialysis) to TSH ratio

Sensitivity is the percentage of cases which are actually positive that are detected as positive by the test.
Specificity is the percentage of euthyroid dogs that have values within the reference interval.
Accuracy is the percentage of all cases with true positive and true negative test results (Chapter 13).

References: Peterson et al., 1997; Dixon and Mooney, 1999. If only one number is listed, the datum is from Peterson et al., 1997.* 100% sensitivity when patients with positive anti-T4 autoantibodies were removed from consideration (Dixon and Mooney, 1999).

d. TSH stimulation test procedure
(1) A blood sample is drawn for baseline T4 determination.
(2) Generally, 0.1 unit TSH/kg body weight is injected and asecond blood sample for T4 determination is drawn six hours later.
(3) Alternatively in dogs, one unit TSH/dog is injected and a second blood sample for T4 determination is drawn four hours later.

e. Interpretation of TSH stimulation test
(1) In euthyroid (healthy) animals, a two-fold elevation of the serum T4 concentration above baseline values should be apparent in the second serum sample using the above protocol.
(2) An alternative interpretation guideline is that the serum T4 concentration in the post-TSH serum sample should increase by at least 2 µg/dL (25 nmol/L) or exceed the upper limit of the reference interval in the euthyroid state.

3. TRH stimulation test
a. In the diagnosis of hypothyroidism, TRH stimulation, with measurement of pre- and post-TRH T4 concentrations, has been used to evaluate pituitary and thyroid secretory reserve.
b. The TRH stimulation test is used most commonly to diagnose hyperthyroidism in cats (see below).
c. TRH stimulation test procedure
(1) A blood sample is drawn for baseline determination of serum T4 concentration.
(2) TRH is administered by injection at a dosage of 200 or 250 µg in dogs or 100 µg/kg in cats.
(3) A second blood sample is drawn four4 hours later for determination of serum T4 concentration.
d. Interpretation of the TRH stimulation test

www.Ebook777.com

(1) The TRH stimulation test often shows only marginal elevation of serum T4 concentrations in euthyroid dogs.
(2) Thyroid autonomy (lack of response to negative feedback control) and hyperthyroidism are considered to exist if the serum T4 concentration fails to increase by 50% following the administration of TRH.

e. The TRH stimulation test has shown poor discrimination between hyperthyroid cats and cats with nonthyroidal illness.

K. TSH response to TRH. Although the increased percentage of change in serum TSH concentration in response to TRH administration can be used to differentiate euthyroid from hypothyroid dogs, this test has little advantage over measurement of baseline serum TSH concentrations because at the time of diagnosis, TSH is being secreted maximally in most dogs.

II. HYPOTHYROIDISM (CASE 28)

A. Incidence of hypothyroidism

1. Hypothyroidism occurs commonly in dogs. It usually is associated with thyroid atrophy or lymphocytic thyroiditis.
2. Hypothyroidism in cats is almost always an iatrogenic disease that follows treatment for hyperthyroidism.
3. Familial hypothyroidism has been reported in Giant Schnauzers and Abyssinian cats.
4. Hypothyroidism has been observed occasionally in horses.

B. Regardless of the animal species, T4 measurements often are inadequate for diagnosis of hypothyroidism.

1. If the serum T4 concentration is within the reference interval, hypothyroidism can be excluded except in approximately 10% of individuals in which anti-T4 autoantibodies exist and lead to a falsely elevated T4 value.
2. If the serum T4 concentration is decreased, serum TSH and/or fT4 concentrations should be measured.
3. An increased serum TSH concentration adds specificity to the diagnosis.
4. A decreased serum fT4 by dialysis concentration is highly specific for hypothyroidism in non-sighthounds
5. Hypothyroidism preferably should be diagnosed only in animals presenting with clinical signs consistent with this disease.

C. Thyroid function testing in birds

1. The circulating half-life (T 1/2) of thyroid hormones in birds is much shorter than in mammals. Accurate determination of thyroid hormonal status in birds is difficult when only a single serum sample is evaluated.
2. Diurnal and seasonal fluctuations in thyroid hormone occur in birds; therefore, the interpretation of thyroid function tests is difficult.
3. Resting thyroid hormone or TSH response is used to evaluate the thyroid status.
4. Standardization of the TSH response test in individual laboratories is necessary to interpret test results reliably.
5. TSH response test protocol
   a. A blood sample is drawn for determination of baseline serum T4 concentration.
   b. 0.1 IU of TSH is given intramuscularly.
   c. A second blood sample is drawn six hours after TSH administration.
   d. Serum T4 concentrations are determined by commercial radioimmunoassay.
   e. The serum T4 concentrations are interpreted as in other animal species.

Free ebooks ==> www.Ebook777.com

D. Secondary hypothyroidism is an uncommon disease in which TSH secretion is inadequate, usually due to a pituitary lesion.

1. Inadequate stimulation of the thyroid gland by TSH results in glandular atrophy.

2. Complete recovery of thyroid axis function generally requires six weeks following adequate thyroxine replacement therapy.

3. Indirect evidence suggests that pituitary TSH release is suppressed with glucocorticoid administration.

E. Common laboratory abnormalities in hypothyroidism include the following:

1. Normocytic, normochromic, nonregenerative anemia occurs in approximately 50% of affected dogs.

2. Hypercholesterolemia is present in approximately 80% of affected dogs.

3. Serum creatine kinase activity is increased in approximately 10% of affected dogs.

4. Experimental hypothyroidism in dogs caused a significant decrease in GFR without altering plasma creatinine concentrations likely because creatinine production was reduced. These results imply that some measurement of GFR might be necessary to evaluate renal function in a hypothyroid patient.

III. HYPERTHYROIDISM (CASE 27)

A. Hyperthyroidism occurs commonly in older cats with nodular (adenomatous) thyroid hyperplasia or thyroid adenomas (Figure 11.3).

B. Hyperthyroidism is uncommon in dogs. It usually is associated with thyroid neoplasia, but most canine thyroid neoplasms are nonfunctional.

C. Common laboratory abnormalities in hyperthyroidism include the following:

1. Polycythemia occurs in approximately 50% of affected cats.

2. Increased serum activity of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase occurs in approximately 50% to 66% of affected cats. Some studies indicate that feline hyperthyroidism is associated with increased serum activity of the bone isoenzyme of alkaline phosphatase, whereas other studies indicate that serum activity of the hepatic isoenzyme of alkaline phosphatase is increased.

3. Renal function

a. Treatment of hyperthyroidism in some patients may result in a mild to severe exacerbation of azotemia, because cardiac output and glomerular filtration declines with re-establishment of euthyroidism.

b. Serum creatinine concentration may be elevated because both chronic renal disease (CRD) and hyperthyroidism are diseases of old age. Furthermore, hyperthyroidism leads to muscle catabolism with the generation of additional creatinine.

c. Hyperthyroidism may mask CRD and it has become a concern to predict cats which might develop overt renal insufficiency following treatment of hyperthyroidism.

(1) Urinary protein/creatinine (UPC) ratios may be elevated (greater than 0.5) in affected cats, but the UPC does not predict which cats will develop azotemia following treatment. However, UPC ratios decline significantly with treatment for hyperthyroidism.

(2) Measurements of tubular function, such as urinary retinol binding protein/creatinine ratio, provided no additional predictive value.

(3) Pre-treatment measurement of GFR, USG, and serum TT4 were shown to have possible predictive value regarding the development of post-treatment renal azotemia.

(4) Renal function changes generally are seen within four weeks of treatment of hyperthyroidism, with no further progression thereafter.

www.Ebook777.com

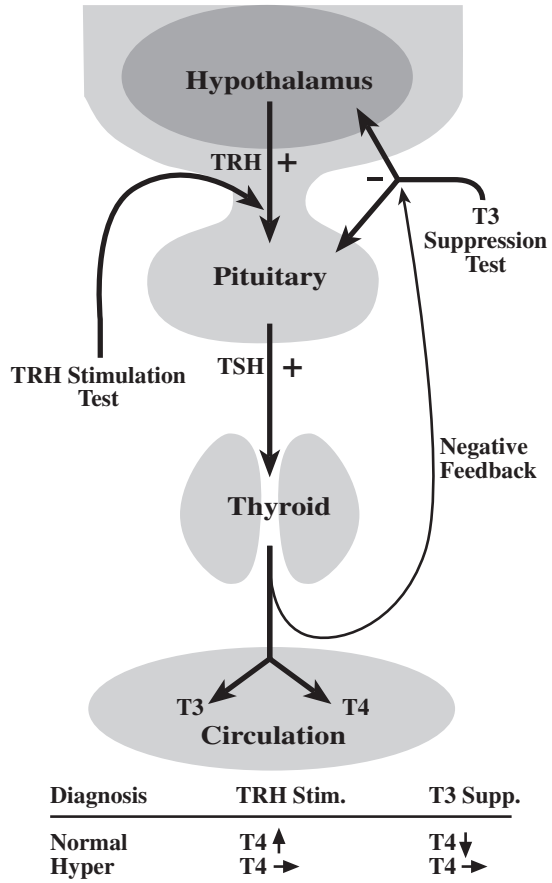


FIGURE 11.3. Overview of function tests in the diagnosis of feline hyperthyroidism. The TRH stimulation test and T3 suppression test evaluate thyroid glandular autonomy. When an appropriate dosage of TRH is given intravenously, the T4 concentration is expected to increase in the healthy euthyroid animal because TRH stimulates TSH release from the pituitary, which in turn stimulates the thyroid glands to secrete T4. In hyperthyroidism, the T4 concentration does not increase significantly. Conversely, if an appropriate dosage of L-T3 is given to a healthy euthyroid animal, the serum T4 concentration should decrease because T3 suppresses TSH release by the pituitary. In hyperthyroidism, T4 secretion is unaffected by T3 administration, presumably because TSH is already suppressed.

4. Glucose and glucose tolerance tests
   - **a.** Some cats with hyperthyroidism are glucose-intolerant or have overt diabetes mellitus. However, treatment of hyperthyroidism does not generally reverse this abnormality, and may exacerbate it.
   - **b.** Fasting hyperglycemia may be present because glucose elimination rates are reduced. Insulin response to glucose also is delayed.
5. Serum fructosamine concentration
   - **a.** Serum fructosamine concentrations of hyperthyroid cats are significantly lower than values in healthy cats due to accelerated plasma protein (albumin) turnover.
   - **b.** Hyperthyroid cats with diabetes mellitus may have serum fructosamine concentrations that may fall within or below the euglycemic reference interval of normal cats.
   - **c.** Serum fructosamine concentration should not be used to diagnose or assess the adequacy of treatment of diabetes in cats with concurrent hyperthyroidism that has not been controlled for at least six weeks.

Free ebooks ==> www.Ebook777.com

D. Diagnosis of hyperthyroidism
- **1.** Serum total T4 concentration is the screening test of choice for hyperthyroidism.
- **2.** Serum total T4 concentration is elevated in the majority of animals with hyperthyroidism.
- **3.** Serum total T4 values may be within the reference interval in mild hyperthyroidism and with nonthyroidal illness.
- **4.** Cats with mild hyperthyroidism may have serum total T4 concentrations that remain within the high end of the reference interval or fluctuate in and out of the high end of the reference interval.

E. Additional tests to confirm hyperthyroidism when serum total T4 concentration is within the reference interval
- **1.** Free T4 (fT4) by dialysis
  - **a.** This test identifies most cats with mild hyperthyroidism or nonthyroidal illness, but has a false-positive rate of approximately 5% to 6% in euthyroid cats.
  - **b.** Cats with severe obesity may develop increased fT4 by dialysis, but test values are usually within the upper end of the reference interval.
  - **c.** The fT4 concentration should be interpreted with knowledge of clinical signs and serum total T4 concentration.
- **2.** T3 suppression test
  - **a.** Serum T4 concentration is measured in blood samples taken before and after exogenous T3 (25 µg given intramuscularly) is administered for seven doses over 48 hours.
  - **b.** The final blood sample is drawn four hours after the seventh dose of exogenous T3.
  - **c.** In healthy cats, the serum T4 concentration decreases by at least 50%.
  - **d.** In hyperthyroid cats, little or no change occurs in serum T4 concentration (Figure 11.1).
- **3.** TRH stimulation test
  - **a.** Blood samples for serum total T4 determination are collected before and after intravenous administration of 0.1 mg/kg of TRH.
  - **b.** Thyroid autonomy (lack of response to negative feedback control) and hyperthyroidism are suspected if the serum T4 concentration fails to increase by 50% following TRH administration (Figure 11.2).
  - **c.** In severely ill cats, the TRH stimulation test may not routinely identify patients with hyperthyroidism because serum T4 concentrations do not increase markedly as anticipated.
  - **d.** The TRH stimulation test in the horse is performed as follows: Administer 1 mg of TRH intravenously and then collect serum samples four and four hours after administration. of TRH. Serum levels of T3 and T4 in normal horses will be at least twice that of baseline after two and four hours.

ENDOCRINE PANCREAS

In addition to its digestive functions, the pancreas secretes hormones that regulate glucose, lipid, and protein metabolism. Diabetes mellitus and hyperinsulinism, associated with abnormalities of the islets of Langerhans, require laboratory evaluation for disease diagnosis and therapeutic management. These diseases occur most often in the dog and cat and only rarely in other domestic animals.

I. BASIC CONCEPTS OF ENDOCRINE PANCREATIC HORMONES

A. Metabolic effects of insulin
- **1.** Beta cells of the islets of Langerhans secrete insulin.
- **2.** The major physiological stimulus for insulin secretion is glucose; however, other nutrients (amino acids and fatty acids) and hormones also are involved in insulin release.
- **3.** Insulin target organs are primarily liver, skeletal muscle, and fat.

www.Ebook777.com

4. Insulin promotes anabolic metabolism of carbohydrates, fats, proteins, and nucleic acids by potentiating the cellular uptake of glucose, other monosaccharides, some amino acids, fatty acids, potassium, and magnesium.
5. Insulin is not required for glucose uptake by erythrocytes, neurons, enterocytes, pancreatic beta cells, renal tubular cells, and ocular lens.

B. Metabolic effects of glucagon
1. Alpha cells of the islets of Langerhans secrete glucagon in response to hypoglycemia, increased plasma concentration of certain amino acids, or certain hormones.
2. The major effects of glucagon are to promote hepatic glycogenolysis and gluconeogenesis, thereby increasing blood glucose concentration.
3. Glucagon also causes lipolysis and ketogenesis. Ketone bodies are used as alternative fuel when cellular glucose uptake is low (e.g., starvation, diabetes mellitus).

C. Metabolic activity of other hormones that oppose the effects of insulin
1. Glucocorticoids antagonize insulin effects by promoting gluconeogenesis, glycogenesis, and lipolysis, and by inhibiting muscular protein synthesis.
2. Catecholamines increase glycogenolysis, gluconeogenesis, and lipolysis.
3. Growth hormone inhibits glucose uptake by insulin-responsive cells and promotes lipolysis.
4. Delta cells of the islets of Langerhans produce somatostatin, which inhibits glucagon and insulin secretion.
5. Progesterone, secreted in high concentration during late estrus and metestrus, may cause excessive growth hormone release from hyperplastic ductular epithelium in the mammary gland of dogs and thereby exert an anti-insulin effect.

II. LABORATORY ASSESSMENT OF THE ENDOCRINE PANCREAS

A. Diabetes mellitus (Cases 15 and 34)
1. Diabetes mellitus results when insulin secretion is inadequate or when tissues fail to respond normally to insulin.
2. Although various types of diabetes mellitus have been described based on glucose tolerance testing and insulin responses, these classifications are not used widely in veterinary medicine.
3. Most diabetic dogs and cats have an absolute deficiency of insulin.
4. Some diabetic cats have normal to increased serum insulin concentrations with peripheral insulin resistance.
5. Diagnosis of diabetes mellitus usually is made on the basis of persistent fasting hyperglycemia and glucosuria.
6. Documentation that hyperglycemia is caused by diabetes mellitus usually can be accomplished by repeated glucose measurements.
   a. Most other causes of hyperglycemia are transient.
   b. Diagnosis of diabetes mellitus can be made with one blood or urine sample if hyperglycemia and ketonemia, or glucosuria and ketonuria, respectively, are present.
7. Laboratory measurement of serum glucose, considerations in sample handling, and general interpretation of hyperglycemia and hypoglycemia are described in Chapter 6.
8. Glucose tolerance tests can be used to help confirm or exclude a diagnosis of diabetes mellitus in animals with equivocal blood glucose test results.
   a. The glucose tolerance test is described in detail in Chapter 6.
   b. This test is labor-intensive and is not performed in most practice settings.
9. Other laboratory abnormalities in diabetes mellitus may include the following:
   a. Ketonemia, ketonuria, and ketoacidosis occur with low blood insulin concentrations (Chapter 6, Case 34).

Free ebooks ==> www.Ebook777.com

b. Lipemia is common (Chapter 6).
c. Osmotic diuresis may produce progressive dehydration and electrolyte loss.
d. Proteinuria may occur due to lower urinary tract infection and/or glomerular disease.
e. Increased glycosylation of hemoglobin (gHb) and serum proteins (measured as fructosamine) occurs and their concentrations may be used as time-averaged indices of blood glucose concentration.
(1) gHb concentration reflects the blood glucose concentration over the previous two to three months.
(2) Fructosamine provides an index of blood glucose concentration over the previous two to three weeks.

B. Hyperinsulinism
1. Functional pancreatic β-cell tumors occasionally cause persistent or periodic hypoglycemia in dogs related to hypersecretion of insulin. However, pancreatic β-cell tumors may secrete other hormones in addition to insulin.
2. Serum insulin concentration is measured to establish a diagnosis of hyperinsulinism, presumably due to β-cell neoplasia.
3. Insulin concentrations must be interpreted in conjunction with serum glucose concentrations.
a. Serum glucose is measured initially without fasting.
b. If the glucose concentration is below 60 mg/dL, insulin can be measured on the same serum sample if it has been stored frozen.
c. If the glucose concentration is greater than 60 mg/dL, the dog is fasted and serum glucose is measured at two-hour intervals until the serum glucose drops below 60 mg/dL.
d. When serum glucose is below 60 mg/dL, serum glucose and insulin are measured on the same sample.
e. If hypoglycemia does not develop after eight to 10 hours of fasting, the test usually is terminated.
4. One widely used technique for evaluating serum glucose and insulin results in dogs with possible hyperinsulinism is the amended insulin/glucose ratio.
a. $\text{Amended insulin/glucose ratio} = \text{serum insulin } (\mu\text{U/ml}) \times 100 \div \text{serum glucose } (\text{mg/dL}) - 30$.
b. Ratios below 30 are considered normal for dogs.
c. Ratios above 30 are suggestive of hyperinsulinism.

ADRENAL CORTEX

Two primary diseases affect the adrenal gland: hyperadrenocorticism and adrenal insufficiency. Hyperadrenocorticism is separated into three types: pituitary-dependent (Cushing's syndrome), adrenal-dependent, and iatrogenic. Laboratory tests are used to confirm the diagnosis of adrenal insufficiency and hyperadrenocorticism, as well as to distinguish between the types of hyperadrenocorticism.

I. BASIC CONCEPTS

A. Glucocorticoid secretion and function
1. The glucocorticoids (cortisol, corticosterone, and cortisone) are secreted by the zona fasciculata and zona reticularis of the adrenal cortex.
2. Glucocorticoid secretion is stimulated by adrenocorticotropic hormone (ACTH), which is released from the anterior pituitary under stimulation of corticotropin-releasing hormone (CRH) from the hypothalamus.

www.Ebook777.com

3. Glucocorticoid secretion is circadian in some species, although a distinct cyclical pattern has been difficult to demonstrate in the dog.
4. Cortisol inhibits release of CRH, suppressing ACTH secretion. It also can suppress ACTH directly. These interactions provide negative feedback regulation of plasma cortisol concentration.
5. Exogenous glucocorticoid administration also suppresses ACTH secretion.
6. Glucocorticoids have major dose-dependent effects on intermediary metabolism.
   a. Glucocorticoids antagonize the effects of insulin. They promote gluconeogenesis and glycogenesis while decreasing glucose uptake by insulin-sensitive tissues.
   b. Glucocorticoids increase lipolysis.
   c. Other actions of glucocorticoids include suppression of wound healing, inflammation, and immunologic responsiveness.

B. Mineralocorticoid secretion and function
1. Aldosterone is the primary mineralocorticoid secreted by the zona glomerulosa of the adrenal cortex.
2. Aldosterone secretion is regulated by several complex mechanisms involving renin, ACTH, and direct stimulation by rising serum potassium (K^+) concentration. The renin-angiotensin system is the primary control mechanism.
3. The kidney is the primary target organ of aldosterone.
   a. Aldosterone promotes renal tubular reabsorption of sodium (Na^+).
   b. Aldosterone promotes renal tubular excretion of K^+.

C. The zona reticularis of the adrenal cortex secretes androgens and estrogens, which may relate to certain clinical features of adrenocortical disease.

II. DISEASES OF THE ADRENAL CORTEX

A. Hyperadrenocorticism (Cases 26 and 34)
1. Pituitary-dependent hyperadrenocorticism is the most common form of naturally occurring canine hyperadrenocorticism.
   a. Pituitary-dependent hyperadrenocorticism results from hyperplasia or small neoplasms of the ACTH-secreting cells of the anterior pituitary.
   b. ACTH excess also may arise from the pars intermedia of the pituitary.
   c. Chronic, excessive ACTH stimulation results in bilateral adrenal cortical hyperplasia.
2. Adrenal-dependent hyperadrenocorticism results from functional adrenal cortical neoplasms that autonomously secrete excessive cortisol. Most of these adrenocortical neoplasms are unilateral.
3. Iatrogenic hyperadrenocorticism occurs in animals receiving long-term glucocorticoid therapy. The clinical signs of iatrogenic hyperadrenocorticism are indistinguishable from those of naturally occurring hyperadrenocorticism.
4. Hyperadrenocorticism is rare in cats; however, both pituitary-dependent and adrenal-dependent forms of the disease are recognized.
5. Hyperadrenocorticism may be a component of a complex endocrinopathy in horses with adenomas of the pars intermedia of the pituitary.
6. Hypercortisolemia, in extremely rare cases, may be caused by ectopic ACTH secretion by non-pituitary or adrenal tumors, or may be food-dependent.

B. Hypoadrenocorticism
1. Hypoadrenocorticism usually results from adrenal gland failure (adrenal-dependent hypoadrenocorticism, Addison's disease) rather than failure of ACTH secretion by the pituitary gland (pituitary-dependent hypoadrenocorticism); however, the specific cause of disease may be undetermined.

Free ebooks ==> www.Ebook777.com

a. Signs of both mineralocorticoid and glucocorticoid deficiency are seen.
b. Immune-mediated destruction of the adrenal cortex has been demonstrated in people and is suspected to occur in dogs.

2. Inadequate ACTH stimulation resulting from pituitary disease is an occasional cause of glucocorticoid deficiency.

3. Chronic or high-dose glucocorticoid therapy may cause adrenal cortical atrophy which may result in iatrogenic hypoadrenocorticism when therapy is stopped suddenly. Signs of glucocorticoid but not mineralocorticoid deficiency are seen.

4. Overzealous therapy of hyperadrenocorticism with o,p'-DDD (Lysodren®) can produce glucocorticoid deficiency and, rarely, mineralocorticoid deficiency. Clinically significant mineralocorticoid deficiency also can be produced with trilostane therapy, although this is rare.

III. LABORATORY EVALUATION OF THE ADRENAL CORTEX (FIGURE 11.4)

A. Plasma cortisol measurement

1. Immunoassays to quantitate cortisol concentrations of plasma have been validated for dogs, cats, and horses. In birds, corticosterone is the principal glucocorticoid secreted by the adrenal gland.

2. In most laboratory assays, cortisol is the primary glucocorticoid that is detected; there is minimal cross-reactivity with corticosterone and cortisone.

3. Prednisolone and other exogenous steroids except dexamethasone will cross-react in commonly used cortisol immunoassays. The degree of cross-reactivity varies with individual assay kits.

4. Resting or baseline cortisol values of animals with hyperadrenocorticism may be within the reference interval.

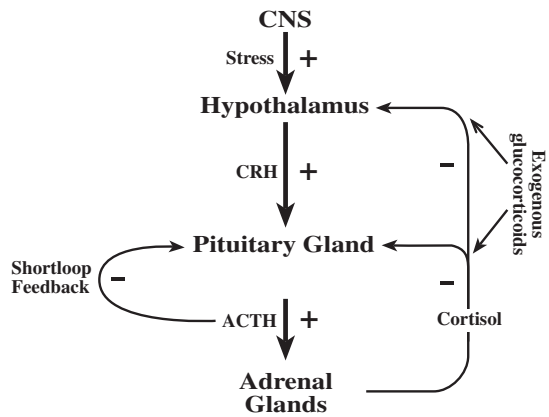


FIGURE 11.4. Overview of the hypothalamic-pituitary-adrenal (HPA) axis and tests of adrenal function. The hypothalamus produces corticotropin releasing hormone (CRH) in response to central nervous system factors often associated with stress. CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary corticotroph cells. ACTH stimulates adrenal gland growth and secretion of cortisol in most domestic mammals (corticosterone is the main product in birds). The unbound or free fraction of cortisol in plasma provides negative feedback regulation of both the pituitary and hypothalamus. Exogenous glucocorticoids, such as dexamethasone, are used in carefully defined dosages (e.g., low-dose dexamethasone suppression test) to evaluate the HPA axis negative feedback systems which become abnormal in pituitary-dependent hyperadrenocorticism. Appropriate dosages of ACTH analogues are administered to evaluate adrenal gland secretory reserve. The adrenal gland secretory reserve is decreased in adrenal insufficiency and generally increased in hyperadrenocorticism and chronic, stressful illnesses.

www.Ebook777.com

5. Cortisol values after adrenal stimulation with exogenous ACTH or after administration of dexamethasone to suppress pituitary-derived endogenous ACTH are used in conjunction with baseline cortisol values to diagnose adrenocortical dysfunction. Several testing protocols and guidelines for interpretation of cortisol values have been suggested.

B. ACTH stimulation test

1. This test evaluates the ability of the adrenal gland to increase plasma cortisol concentration in response to ACTH stimulation.
2. ACTH stimulation test protocol
   a. A blood sample is drawn for determination of baseline plasma cortisol concentration.
   b. Synthetic ACTH (Cosyntropin®) is administered intravenously. The dosage of synthetic ACTH varies with the animal species.
      (1) 125 µg Cosyntropin® in the cat
      (2) 250 µg Cosyntropin® in the dog
      (3) 1 mg Cosyntropin® in the horse
   c. A second blood sample is drawn post-ACTH administration to determine plasma cortisol concentration following adrenal gland stimulation. The timing of blood sample collection is as follows:
      (1) 60 and 90 minutes post ACTH administration in the cat
      (2) 60 minutes post ACTH administration in the dog
      (3) Two and four hours post ACTH administration in the horse
      (4) These times approximate peak plasma cortisol concentrations after ACTH administration.
3. Interpretation of the ACTH stimulation test
   a. Interpretation of the ACTH stimulation test should be based on absolute values of cortisol concentration in the post ACTH sample rather than relative increases (percentages).
   b. Normal adrenocortical activity in dogs typically exhibits a two- to three-fold increase in plasma cortisol concentration above baseline values.
   c. Pituitary-dependent hyperadrenocorticism in dogs typically produces post-ACTH cortisol concentrations that are greater than 20 µg/dL.
   d. Although individual test results must be interpreted with reference intervals for the laboratory performing the tests, inter-laboratory variations in cortisol measurement by immunoassay usually are minimal. Therefore, published reference intervals for plasma cortisol concentration may provide useful guidelines for interpreting the ACTH stimulation test results.
4. At least 50% of dogs with functional adrenal tumors have abnormal ACTH responses.
5. Some dogs with pituitary-dependent hyperadrenocorticism and a larger proportion of dogs with functional adrenal tumors may have a normal response to the ACTH stimulation test.
6. Dogs with iatrogenic hyperadrenocorticism have little or no response to exogenous ACTH administration. This is the test of choice to diagnose iatrogenic hyperadrenocorticism.
7. Dogs with naturally-occurring hypoadrenocorticism have little or no response to exogenous ACTH administration. The ACTH stimulation test does not allow identification of the cause of hypoadrenocorticism (pituitary vs. adrenal vs. iatrogenic).

C. Low-dose dexamethasone suppression test (Case 34)

1. This test is used to screen animals for pituitary-dependent and adrenal-dependent hyperadrenocorticism.
2. Low-dose dexamethasone suppression test protocol
   a. A blood sample is drawn for baseline plasma cortisol determination.
   b. Dexamethasone is injected intraveneously at the following dosages:
      (1) 0.01 mg/kg in the dog
      (2) 0.1 mg/kg in the cat
   c. A second blood sample is drawn eight hours after administration of dexamethasone for determination of plasma cortisol concentration.

Free ebooks ==> www.Ebook777.com

3. Interpretation of the low-dose dexamethasone suppression test
   a. Cortisol secretion is markedly inhibited by dexamethasone; healthy dogs and cats have eight-hour cortisol concentrations less than 1 ug/dL.
   b. In dogs with either pituitary-dependent or adrenal-dependent hyperadrenocorticism, plasma cortisol concentrations usually are inadequately suppressed at eight hours post-dexamethasone administration.
   c. The low-dose dexamethasone suppression test is considered sensitive because only about 5% of dogs with naturally occurring hyperadrenocorticism have normal test results.
   d. Abnormal low-dose dexamethasone suppression test results may be seen with stress.

D. High-dose dexamethasone suppression test (Case 34)
1. This test is used to distinguish dogs with pituitary-dependent hyperadrenocorticism from dogs with adrenal-dependent hyperadrenocorticism.
2. High-dose dexamethasone suppression test protocol
   a. A blood sample is drawn for baseline plasma cortisol determination.
   b. Dexamethasone is injected intraveneously at the following dosages:
      (1) 0.1 to 1.0 mg/kg in the dog
      (2) 1.0 mg/kg in the cat
   c. A second blood sample is drawn eight hours after administration of dexamethasone for determination of plasma cortisol concentration.
3. Interpretation of the high-dose dexamethasone suppression test
   a. In normal dogs and cats, the cortisol concentration in the eight-hour sample is suppressed to the low limit of detection of the cortisol assay.
   b. Most dogs with pituitary-dependent hyperadrenocorticism also suppress to this extent.
   c. Inadequate suppression of plasma cortisol concentration is observed in most dogs with adrenal tumors and in approximately 25% of dogs with pituitary-dependent hyperadrenocorticism.

E. Urine cortisol/creatinine ratio
1. The urine cortisol/creatinine ratio has been advocated as a screening test for hyperadrenocorticism.
2. An increased cortisol/creatinine ratio is a sensitive indicator of naturally occurring hyperadrenocorticism; however, increased ratios also may occur in dogs that are stressed.
3. The urine cortisol/creatinine ratio attempts to offset within-day fluctuations in plasma cortisol concentration. This is based on the premise that cortisol concentration in a randomly collected urine sample reflects the average plasma cortisol during the time in which that urine was produced.
4. Cortisol and creatinine concentrations are measured concurrently in a randomly collected urine sample.
5. Because of variations in the urine cortisol assay, it is particularly important to use reference values from the laboratory performing the test.
6. The urine cortisol/creatinine ratio cannot be used to monitor treatment with trilostane of dogs with hyperadrenocorticism.

F. Urinary cortisol/creatinine ratio with high-dose suppression test
1. This test is used to differentiate between pituitary-dependent and adrenal-dependent hyperadrenocorticism, but is infrequently performed.
2. Urinary cortisol and creatinine concentrations are measured in samples from three consecutive mornings. After collection of the second urine sample, three oral doses of 0.1 mg/kg dexamethasone are administered at eight-hour intervals.
3. If the ratio of the third urine sample is more than 50% lower than the mean of the first two samples, pituitary-dependent hyperadrenocorticism is probably present.
4. Reference intervals from the laboratory performing the test should be used to interpret the test results.

www.Ebook777.com

G. Measurement of endogenous ACTH concentration

1. Measurement of endogenous ACTH concentration by radioimmunoassay may be useful in distinguishing pituitary-dependent forms of hyperadrenocorticism from adrenal-dependent forms. Plasma concentrations were very high in a dog with ectopic ACTH secretion and low in a dog with food-dependent glucocorticoid excess.

2. Interpretation of endogenous ACTH concentrations

a. Plasma ACTH concentration is within the reference interval or high in pituitary-dependent hyperadrenocorticism, whereas plasma ACTH concentration is low in adrenal-dependent hyperadrenocorticism.

b. Plasma ACTH concentration is decreased in dogs with pituitary-dependent hypoadrenocorticism, whereas plasma ACTH concentration is increased in dogs with adrenal-dependent hypoadrenocorticism.

3. Measurement of endogenous ACTH concentration in animals has been limited by technical difficulties including questionable stability of ACTH in the plasma and hormone interaction with plastic and glass tubes.

H. Other laboratory abnormalities in hyperadrenocorticism

1. Other laboratory abnormalities may include the following:

a. Leukocytosis may be characterized by neutrophilia, lymphopenia, and eosinopenia. In some animals with chronic disease, the neutrophil count may return to the reference interval.

b. Serum alkaline phosphatase activity frequently is increased in affected dogs.

(1) Both the hepatic and the steroid-induced isoenzymes of alkaline phosphatase may increase (see Chapter 7).

(2) Serum activity of the steroid isoenzyme may be increased markedly. Determination of steroid isoenzyme activity has been suggested as a useful screening test for hyperadrenocorticism.

c. Hyperglycemia is sometimes present.

(1) In dogs, the magnitude of hyperglycemia usually does not exceed the renal threshold.

(2) In horses, the magnitude of hyperglycemia exceeds the renal threshold and glucosuria is common.

d. Lipemia and increased serum cholesterol concentration may be observed.

e. The urine is dilute; the specific gravity typically indicates isosthenuria or hyposthenuria.

2. Abnormalities in plasma cortisol concentration in pituitary-dependent hyperadrenocorticism with bilateral adrenocortical hyperplasia

a. The baseline plasma cortisol concentration may be within the reference interval or elevated.

b. Most affected dogs exhibit an exaggerated plasma cortisol response to ACTH stimulation; however, a few affected dogs have plasma cortisol values within the reference interval.

c. Low doses of dexamethasone fail to suppress plasma cortisol concentrations in horses and most dogs and cats with pituitary-dependent hyperadrenocorticism.

d. High doses of dexamethasone suppress plasma cortisol concentrations in most dogs with pituitary-dependent hyperadrenocorticism.

e. High doses of dexamethasone fail to suppress plasma cortisol concentrations in all horses and a low percentage of dogs with pituitary-dependent hyperadrenocorticism.

3. Abnormalities in plasma cortisol concentration in adrenal-dependent hyperadrenocorticism with a functional adrenocortical neoplasm and atrophy of the contralateral adrenal gland

a. Baseline plasma cortisol concentration may be within the reference interval or increased.

b. The plasma cortisol response to ACTH stimulation is within the reference interval or exaggerated.

c. The plasma cortisol concentration fails to suppress with either low or high doses of dexamethasone.

Free ebooks ==> www.Ebook777.com

4. Abnormalities in plasma cortisol concentration in iatrogenic hyperadrenocorticism with bilateral adrenocortical atrophy
   a. The baseline plasma cortisol concentration is within the reference interval or decreased.
   b. There is no response to administration of ACTH.

I. Laboratory findings in adrenal insufficiency with cortical atrophy or an absence of histologic lesions in the adrenal gland
1. Plasma ACTH concentration and attempted ACTH stimulation
   a. Baseline plasma cortisol concentration may be within the reference interval or decreased. A dose of 5 µg/kg has been shown to be equivalent to 250 µg/dog to distinguish between nonadrenal illness and hypoadrenocorticism in dogs
   b. There is no response to attempted ACTH stimulation.
2. Other laboratory abnormalities may include the following:
   a. Hyponatremia and hyperkalemia develop from renal loss of Na^+ and retention of K^+ because of aldosterone deficiency.
      (1) Na^+/K^+ ratio less than 23:1 is highly suggestive of adrenal insufficiency.
      (2) Na^+/K^+ ratio less than 26:1 also may be suggestive of adrenal insufficiency.
      (3) Sodium and potassium values that are within the reference interval do not exclude adrenal insufficiency.
   b. Hypercalcemia may be present.
   c. Lymphocytosis may be observed in a minor proportion of animals with adrenal insufficiency
   d. Hypoglycemia may be observed. Hyperglycemia may be present with concurrent diabetes mellitus.
   e. The adrenal glands may have cortical atrophy or lesions may not be present.

REFERENCES

Angles JM, Feldman EC, Nelson RW, et al: 1997. Use of urine cortisol:creatinine ratio versus adrenocorticotrophic hormone stimulation testing for monitoring mitotane treatment of pituitary-dependent hyperadrenocorticism in dogs. *J Am Vet Med Assoc* 211:1002–1004.

Barber PJ, Elliott J: 1998. Feline chronic renal failure: Calcium homestasis in 80 cases diagnosed between 1992 and 1995. *J Small Anim Pract* 39:108–116.

Becker TJ, Graves TK, Kruger JM, et al: 2000. Effects of methimazole on renal function in cats with hyperthyroidism. *J Am Anim Hosp Assoc* 36:215–223.

Chew DJ, Carothers M: 1989. Hypercalcemia. *Vet Clin North Am Small Anim Pract* 19:265–287.

DeVries SE, Feldman EC, Nelson RW, et al: 1993. Primary parathyroid gland hyperplasia in dogs: Six cases (1982–1991). *J Am Vet Med Assoc* 202:1132–1136.

Diaz-Espineira MM, Mol JA, van den Ingh TSGAM, et al: 2008. Functional and morphological changes in the adenohypophysis of dogs with induced primary hypothyroidism: Loss of TSH hypersecretion, hypersomatotropism, hypoprolactinemia, and pituitary enlargement with transdifferentiation. *Domest Anim Endocrinol* 35:98–111.

Dixon RM, Mooney CT: 1999. Canine serum thyroglobulin autoantibodies in health, hypothyroidism and non-thyroidal illness. *Res Vet Sci* 6:243–246.

Drobatz KJ, Hughes D: 1997. Concentration of ionized calcium in plasma from cats with urethral obstruction. *J Am Vet Med Assoc* 211:1392–1395.

www.Ebook777.com

Elliott J, Dobson JM, Dunn JK, et al: 1991. Hypercalcemia in the dog: A study of 40 cases. *J Small Anim Pract* 32:564–571.

Feldman EC, Nelson RW, Feldman MS: 1996. Use of low- and high-dose dexamethasone tests for distinguishing pituitary-dependent from adrenal tumor hyperadrenocorticism in dogs. *J Am Vet Med Assoc* 209:772–775.

Feldman EC, Nelson RW: 2003. Canine and Feline Endocrinology and Reproduction, 3rd ed. WB Saunders Co., Philadelphia.

Ferguson DC: 1995. Free Thyroid Hormone Measurements in the Diagnosis of Thyroid Disease. In: Bonagura J, Kirk RW (eds): Current Veterinary Therapy XII. WB Saunders Co., Philadelphia, pp. 360–363.

Ferguson DC: 1997. Euthyroid sick syndrome. *Canine Pract* 22:49–51.

Ferguson DC: 2000. Advances in interpretation of thyroid function tests. *Adv Small Anim Med* 13:1–3.

Ferguson DC: 2007. Testing for hypothyroidism in dogs. *Vet Clin N Am Small Animal Pract* 37:647–669.

Ferguson DC: 2009. Thyroid Hormones and Antithyroid Drugs. In: Riviere JE, Papich M (eds): Veterinary Pharmacology and Therapeutics, 9th ed. Wiley-Blackwell, New York, pp. 735–770.

Ferguson DC, Dirikolu L, Hoenig M: 2009. Adrenal Corticosteroids, Mineralocorticoids, and Steroid Synthesis Inhibitors. In: Riviere JE, Papich M (eds): Veterinary Pharmacology and Therapeutics, 9th ed. Wiley-Blackwell, New York, pp. 771–802.

Ferguson DC, Freedman, R: 2006, Goiter in Apparently Euthyroid Cats. In: August, JR (ed.): Consultations in Feline Internal Medicine, Vol. 5. Elsevier Saunders, St. Louis, pp. 207–215.

Ferguson DC, Peterson ME: 1992. Serum free and total iodothyronine concentrations in dogs with hyperadrenocorticism. *Am J Vet Res* 53:1636–1640.

Foster DJ, Thoday KL: 2000. Tissue sources of serum alkaline phosphatase in 34 hyperthyroid cats: A qualitative and quantitative study. *Res Vet Sci* 68:89–94.

Galac S, Buijtels JJ, Kooistra HS. 2009. Urinary corticoid:creatinine ratios in dogs with pituitary-dependent hypercortisolism during trilostane treatment. *J Vet Intern Med* 23:1214–1219.

Galac S, Kars VJ, Voorhut G, et al: 2008. ACTH-independent hyperadrenocorticism due to meal-induced hypercortisolemia in a dog. *Vet J* 177:141–143.

Galac S, Kooistra HS, Voorhut G, et al: 2005. Hyperadrenocorticism in a dog due to ectopic secretion of adrenocorticotropic hormone. *Domest Anim Endocrinol* 28:338–348.

Gaskill CL, Burton SA, Gelens HC, et al: 1999. Effects of phenobarbital treatment on serum thyroxine and thyroid-stimulating hormone concentrations in epileptic dogs. *J Am Vet Med Assoc* 215:489–496.

Gaughan KR, Bruyette DS: 2001. Thyroid function testing in Greyhounds. *Am J Vet Res* 62:1130–1133.

Goossens MM, Meyer HP, Voorhout G, et al: 1995. Urinary excretion of glucocorticoids in the diagnosis of hyperadrenocorticism in cats. *Domest Anim Endocrinol* 12:355–362.

Graham PA, Lundquist RB, Refsal KR, et al: 2001. A 12-month prospective study of 234 thyroglobulin antibody-positive dogs which had no laboratory evidence of thyroid dysfunction. *J Vet Intern Med* 15:298 (abstract #105).

Graham PA, Nachreiner RF, Refsal KR, et al: 2001. Lymphocytic thyroiditis. *Vet Clin North Am Small Anim Pract* 31:915–33.

Free ebooks ==> www.Ebook777.com

Greco DS, Peterson ME, Davidson AP, et al: 1999. Concurrent pituitary and adrenal tumors in dogs with hyperadrenocorticism: 17 cases (1978–1995). *J Am Vet Med Assoc* 214:1349–1353.

Greco DS: 2001. Diagnosis of diabetes mellitus in cats and dogs. *Vet Clin North Am Small Anim Pract* 31:845–953.

Grosenbaugh DA, Gadawski JE, Muir WW: 1998. Evaluation of a portable clinical analyzer in a veterinary hospital setting. *J Am Vet Med Assoc* 213:691–694.

Guptill L, Scott-Moncrieff JC, Bottoms G, et al: 1997. Use of the urine cortisol:creatinine ratio to monitor treatment response in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 210:1158–1161.

Harms CA, Hoskinson JJ, Bruyette DS, et al: 1994. Development of an experimental model of hypothyroidism in cockatiels (*Nymphicus hollandicus*). *Am J Vet Res* 55:399–404.

Hoenig M, Ferguson DC: 1999. Diagnostic utility of glycosylated hemoglobin concentrations in the cat. *Domest Anim Endocrinol* 16:11–17.

Hoenig M, Peterson ME, Ferguson D: 1992. Glucose tolerance and insulin secretion in cats with spontaneous hyperthyroidism. *Res Vet Sci* 53:338–341.

Huang HP, Yang HL, Liang SL, et al: 1999. Iatrogenic hyperadrenocorticism in 28 dogs. *J Am Anim Hosp Assoc* 35:200–207.

Iversen L, Jensen AL, Hoier R, et al: 1998. Development and validation of an improved enzyme-linked immunosorbent assay for the detection of thyroglobulin autoantibodies in canine serum samples. *Domest Anim Endocrinol* 15:525–536.

Jensen AL, Iversen L, Koch J, et al: 1997. Evaluation of the urinary cortisol:creatinine ratio in the diagnosis of hyperadrenocorticism in dogs. *J Small Anim Pract* 38:99–102.

Kallet AJ, Richter KP, Feldman EC, et al: 1991. Primary hyperparathyroidism in cats: Seven cases (1984–1989). *J Am Vet Med Assoc* 199:1767–1771.

Kantrowitz LB, Peterson ME, Melian C, et al: 2001. Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. *J Am Vet Med Assoc* 219:765–769.

Kaplan AJ, Peterson ME, Kemppainen RJ: 1995. Effects of disease and the results of diagnostic tests for use in detecting hyperadrenocorticism in dogs. *J Am Vet Med Assoc* 207:445–451.

Kaptein EM, Hays MT, Ferguson DC: 1994. Thyroid hormone metabolism: A comparative evaluation. *Vet Clin North Am Small Anim Pract* 24:431–466.

Kerl ME, Peterson ME, Wallace MS, et al: 1999. Evaluation of a low-dose synthetic adrenocorticotrophic hormone stimulation test in clinically normal dogs and dogs with naturally developing hyperadrenocorticism. *J Am Vet Med Assoc* 214:1497–1501.

Kintzer PP, Peterson ME: 1997. Diagnosis and management of canine cortisol-secreting adrenal tumors. *Vet Clin North Am Small Anim Pract* 27:299–307.

Kogika MM, Lustoza MD, Notomi MK, et al: 2006. Serum ionized calcium in dogs with chronic renal failure and metabolic acidosis. *Vet Clin Pathol* 35:441–445.

Kooistra HS, Diaz-Espineira M, Mol JA, et al: 2000. Secretion pattern of thyroid-stimulating hormone in dogs during euthyroidism and hypothyroidism. *Domest Anim Endocrinol* 18:19–29.

Kooistra HS, Voorhout G, Mol JA, et al: 1997. Correlation between impairment of glucocorticoid feedback and the size of the pituitary gland in dogs with pituitary-dependent hyperadrenocorticism. *J Endocrinol* 152:387–394.

www.Ebook777.com

Lathan P, Moore GE, Zambon S, et al: 2008. Use of a low-dose ACTH stimulation test for diagnosis of hypoadrenocorticism in dogs. *J Vet Intern Med* 22:1070–1073.

Lurye JC, Behrend EN: 2001. Endocrine tumors. *Vet Clin North Am Small Anim Pract* 31:1083–1110.

Lyon ME, Guajardo M, Laha T, et al: 1995. Electrolyte balanced heparin may produce a bias in the measurement of ionized calcium concentration in specimens with abnormally low protein concentration. *Clin Chem Acta* 233:105–113.

Marca MC, Loste A, Orden I, et al: 2001. Evaluation of canine serum thyrotropin (TSH) concentration: Comparison of three analytical procedures. *J Vet Diagn Invest* 13:106–110.

Melian C, Peterson ME: 1996. Diagnosis and treatment of naturally occurring hypoadrenocorticism in 42 dogs. *J Small Anim Pract* 37:268–275.

Merryman JI, Buckles EL: 1998. The avian thyroid gland. Part one: A review of the anatomy and physiology. *J Avian Med Surg* 12:234–237.

Merryman JI, Buckles EL: 1998. The avian thyroid gland. Part two: A review of function and pathophysiology. *J Avian Med Surg* 12:238–242.

Messinger JS, Windham WR, Ward, CR: 2009. Ionized hypercalcemia in dogs: A retrospective study of 109 cases (1998–2003). *J Vet Intern Med* 23:514–519.

Muller PB, Taboada J, Hosgood G, et al: 2000. Effects of long-term phenobarbital treatment on the thyroid and adrenal axis and adrenal function tests in dogs. *J Vet Intern Med* 14:157–164.

Nachreiner RF, Refsal KR, Graham PA, et al: 2002. Prevalence of serum thyroid hormone autoantibodies in dogs with clinical signs of hypothyroidism. *J Am Vet Med Assoc* 220:466–471.

Nachreiner RF, Refsal KR, Graham PA, et al: 1998. Prevalence of autoantibodies to thyroglobulin in dogs with nonthyroidal illness. *Am J Vet Res* 59:951–955.

Nelson R, Bertoy E: 1997. Use of the urine cortisol:creatinine ratio to monitor treatment response in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 210:1158–1161.

Norman EJ, Thompson H, Mooney CT: 1999. Dynamic adrenal function testing in eight dogs with hyperadrenocorticism associated with adrenocortical neoplasia. *Vet Rec* 144:551–554.

Panciera DL, Lefebvre HP: 2009. Effect of experimental hypothyroidism on glomerular filtration rate and plasma creatinine concentration in dogs. *J Vet Intern Med* 23:1045–1050.

Paster MB: 1991. Avian reproductive endocrinology. *Vet Clin North Am Small Anim Pract* 21:1343–1359.

Peterson ME, Gamble DA: 1990. Effect of nonthyroidal illness on serum thyroxine concentrations in cats: 494 cases (1988). *J Am Vet Med Assoc* 197:1203–1208.

Peterson ME, Graves TY, Gamble DA: 1990. Triiodothyronine (T3) suppression tests: An aid in the diagnosis of mild hyperthyroidism in cats. *J Vet Intern Med* 4:233–238.

Peterson ME, James KM, Wallace M, et al: 1991. Idiopathic hypoparathyroidism in five cats. *J Vet Intern Med* 5:47–51.

Peterson ME, Kintzer PP, Kass PH: 1996. Pretreatment clinical and laboratory findings in dogs with hyperadrenocorticism: 225 cases (1979–1993). *J Am Vet Med Assoc* 208:85–91.

Peterson ME, Melian C, Nichols R: 2001. Measurement of serum concentrations of free thyroxine, total thyroxine, and total triiodothyronine in cats with hyperthyroidism and cats with nonthyroidal disease. *Am Vet Med Assoc* 218: 529–536.

Free ebooks ==> www.Ebook777.com

Peterson ME, Melian C, Nichols R: 1997. Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs. *J Am Vet Med Assoc* 211:1396–1402.

Rae M: 1995. Endocrine disease in pet birds. *Semin Avian Exotic Pet Med* 4:32–38.

Randolph JF, Toomey J, Center SA, et al: 1998. Use of the urine cortisol-to-creatinine ratio for monitoring dogs with pituitary-dependent hyperadrenocorticism during induction treatment with mitotane (o,p' DDD). *Am J Vet Res* 59:258–261.

Rayalam S, Hoenig MH, Ferguson DC: 2009. Hypothalamic and Pituitary Hormones. In: Riviere JE, Papich M (eds): Veterinary Pharmacology and Therapeutics, 9th ed. Wiley-Blackwell, New York, NY, pp. 693–716.

Refsal KR, Nachreiner RF, Stein BE, et al: 1991. Use of the triiodothyronine suppression test for diagnosis of hyperthyroidism in ill cats that have serum concentration of iodothyronines within normal range. *J Am Vet Med Assoc* 199:1594–1601.

Reimers TJ, Lawler DF, Sutaria PM, et al: 1990. Effects of age, sex and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. *Am J Vet Res* 51:454–457.

Reine NJ: 2007. Medical management of pituitary-dependent hyperadrenocorticism: Mitotane versus trilostane. *Clin Tech Small Anim Pract* 22:18–25.

Reusch CE, Tomsa K: 1999. Serum fructosamine concentration in cats with overt hyperthyroidism *J Am Vet Med Assoc* 215:1297–1300.

Rodriguez Pineiro MI, Benchekroun G, de Fornel-Thibaud P, et al. : 2009. Accuracy of an adrenocorticotropic hormone (ACTH) immunoluminometric assay for differentiating ACTH-dependent from ACTH-independent hyperadrenocorticism in dogs. *J Vet Intern Med* 23:850–855.

Rosol TJ, Nagode LA, Couto CG, et al: 1992. Parathyroid hormone (PTH)-related protein, PTH, and 1,25-dihydroxyvitamin D in dogs with cancer-associated hypercalcemia. *Endocrinology* 131:1157–1164.

Ross JT, Scavelli TD, Matthiesen DT, et al: 1991. Adenocarcinoma of the apocrine glands of the anal sac in dogs: A review of 32 cases. *J Am Anim Hosp Assoc* 27:349–355.

Schaer M: 2001. A justification for urine glucose monitoring in the diabetic dog and cat. *J Am Anim Hosp Assoc* 37:311–312.

Schenck PA, Chew DJ: 2008. Calcium: Total or ionized? *Vet Clin North Am Small Anim Pract* 38:497–502.

Schenck PA, Chew DJ: 2003. Determination of calcium fractionation in dogs with chronic renal failure. *Am J Vet Res* 64:1181–1184.

Schenck PA, Chew DJ: 2005. Prediction of serum ionized calcium concentration by use of serum total calcium concentration in dogs. *Am J Vet Res* 66:1330–1336.

Scott-Moncrieff CR, Nelson RW, Bruner JM, et al: 1998. Comparison of serum concentrations of thyroid stimulating hormone in healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. *J Am Vet Med Assoc* 212:387–391.

Scott-Moncrieff CR, Nelson RW: 1998. Change in serum thyroid-stimulating hormone concentration in response to administration of thyrotropin-releasing hormone to healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. *J Am Vet Med Assoc* 213:1435–1438.

Smiley LE, Peterson ME: 1993. Evaluation of a urine cortisol:creatinine ratio as a screening test for hyperadrenocorticism in dogs. *J Vet Intern Med* 7:163–168.

www.Ebook777.com

Sterczer A, Meyer HP, Van Sluijs FJ, et al: 1999. Fast resolution of hypercortisolism in dogs with portosystemic encephalopathy after surgical shunt closure. *Res Vet Sci* 66:63–67.

Suave F, Paradis M: 2000. Use of recombinant human thyroid-stimulating hormone for thyrotropin stimulation test in euthyroid dogs. *Can Vet J* 41:215–219.

Syme HM, Elliott J: 2001. Evaluation of proteinuria in hyperthyroid cats. *J Vet Intern Med* 15:299 (abstract #110).

Syring RS, Otto CM, Drobatz KJ: 2001. Hyperglycemia in dogs and cats with head trauma. *J Am Vet Med Assoc* 218:1124–1129.

Teske E, Rothuizen J, de Bruijne JJ, et al: 1989. Corticosteroid-induced alkaline phosphatase isoenzyme in the diagnosis of canine hypercorticism. *Vet Rec* 125:12–14.

Thoday KL, Mooney CT: 1992. Historical, clinical and laboratory features of 126 hyperthyroid cats. *Vet Rec* 131:257–264.

Tomsa K, Glaus TM, Kacl GM, et al: 2001. Thyrotropin-releasing hormone stimulation test to assess thyroid function in severely sick cats. *J Vet Intern Med* 15:89–93.

Toribio RE, Kohn CW, Capen CC, et al: 2003. Parathyroid hormone (PTH) secretion, PTH mRNA and calcium-sensing receptor mRNA expression in equine parathyroid cells, and effects of interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha on equine parathyroid cell function. *J Molec Endocrinol* 31:609–620.

Toribio RE, Kohn CW, Chew DJ, et al: 2001. Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis. *Am J Vet Res* 62:938–947.

Toribio RE, Kohn CW, Chew DJ, et al: 2001. Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis. *Am J Vet Res* 62:938–947.

Torrance AG, Nachreiner R: 1989. Intact parathyroid hormone assay and total calcium concentration in the diagnosis of disorders of calcium metabolism in dogs. *J Vet Intern Med* 3:86–89.

Turrel JM, Feldman EC, Nelson RW, et al: 1988. Thyroid carcinoma causing hyperthyroidism in cats: 14 cases (1981–1986). *J Am Vet Med Assoc* 193:359–364.

van Hoek I, Lefebvre HP, Peremans K, et al: 2009. Short- and long-term follow-up of glomerular and tubular renal markers of kidney function in hyperthyroid cats after treatment with radioiodine. *Domestic Anim Endocrinol* 36:45–56.

van Hoek IM, Vandermeulen E, Peremans K, et al: 2010. Thyroid stimulation with recombinant human thyrotropin in healthy cats, cats with non-thyroidal illness and in cats with low serum thyroxin and azotaemia after treatment of hyperthyroidism. *J Feline Med Surg* 12:117–121.

Von Klopmann T, Boettcher IC, Rotermund A, et al: 2006. Euthyroid sick syndrome in dogs with idiopathic epilepsy before treatment with anticonvulsant drugs. *J Vet Intern Med* 20:516–522.

Wathers CB, Scott-Moncrieff JCR: 1992. Hypocalcemia in cats. *Compend Contin Educ Pract Vet* 14:497–507.

Watson AD, Church DB, Emslie DR, et al: 1998. Plasma cortisol responses to three corticotrophic preparations in normal dogs. *Aust Vet J* 76:255–257.

Whitley NT, Drobatz KJ, Panciera DL: 1997. Insulin overdose in dogs and cats: 28 cases (1986–1993). *J Am Vet Med Assoc* 211:326–330.

Free ebooks ==> www.Ebook777.com

Wood MA, Panciera DL, Berry SH, et al: Influence of isoflurane general anesthesia or anesthesia and surgery on thyroid function tests in dogs. *J Vet Intern Med* 23:7–15, 2009.

Yang X, McGraw RA, Ferguson DC: 2000. cDNA cloning of canine common alpha gene and its co-expression with canine thyrotropin beta gene in baculovirus expression system. *Domest Anim Endocrinol* 18:379–393.

Yang X, McGraw RA, Su X, et al: 2000. Canine thyrotropin beta-subunit gene: Cloning and expression in *Escherichia coli*, generation of monoclonal antibodies, and transient expression in the Chinese hamster ovary cells. *Domest Anim Endocrinol* 18:363–78.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 12

CYTOLOGY

Pauline M. Rakich, DVM, PhD, and Kenneth S. Latimer, DVM, PhD

INTRODUCTION

Cytology is a rapid, simple, and economical diagnostic technique that usually poses minimal risk to the patient. In many cases, a definitive cytologic diagnosis can be made. However, even when a specific diagnosis cannot be rendered, the information provided by cytology (e.g., inflammation vs. neoplasia) is useful for choosing additional tests to make a definitive diagnosis (e.g., culture vs. histopathology).

I. SPECIMEN COLLECTION AND HANDLING

The usefulness of cytology directly depends on the quality of sample collection and preparation.

A. Tissue imprints

1. Touch imprints can be prepared from external lesions or masses as well as from biopsies of deep tissues.

2. Imprints of surface material may reflect only superficial inflammation and infection and may not be representative of deeper changes. However, certain pathogens (e.g., *Dermatophilus congolensis*) may be present only on the tissue surface.

3. The tissue should be blotted to remove blood and tissue fluid which dilute diagnostic material and prevent cells from adhering to the slide and flattening out adequately for examination.

4. A slide is gently pressed against the tissue or lesion one or more times to make the cytologic touch imprints.

B. Tissue scrapings

1. This method is used when impressions would yield too few cells (e.g., conjunctiva, firm tissues) or it is not possible to imprint.

2. A scalpel blade is scraped across the tissue several times until a small amount of material collects on the blade. This material is spread across a slide to make the smear.

3. For conjunctival scrapings, a specially designed spatula (Kimura platinum spatula) or scalpel handle can be used to collect cells. Swabs are not sufficiently abrasive to obtain a diagnostic specimen.

C. Fine-needle aspiration

1. Fine-needle aspiration (FNA) is used for lesions beneath the skin surface or in deep tissues or internal organs.

2. A 21- to 25-gauge needle, attached to a 3- to 12-mL syringe, is inserted into the tissue, negative pressure is applied by withdrawing the plunger to approximately three-fourths of the syringe volume, and the needle is redirected several times to sample multiple areas of the tissue or mass.

www.Ebook777.com

3. Unless the mass is filled with fluid, aspirated material will be present in the needle but will not be visible in the syringe.
4. If blood is seen in the syringe, negative pressure should be released immediately to prevent dilution of the aspirated material with blood cells.
5. Before the needle is removed from the tissue, negative pressure is released to prevent contamination of the sample with cells from surrounding tissues and skin and to prevent loss of aspirated material into the syringe.
6. The needle is then removed from the syringe, air is drawn into the syringe, the needle is reconnected and placed against a glass slide, and the aspirated material within the needle is expressed onto the slide.
7. The material is smeared out to prepare a monolayer of flattened cells.

D. Stab method/nonaspiration fine-needle biopsy
1. Specimens normally sampled by FNA also can be obtained by a needle inserted into the lesion/tissue without use of negative pressure.
2. The (22-gauge to 25-gauge) needle is introduced into the tissue and gently moved up and down the needle tract to harvest cells. The material in the needle is expelled onto a slide and smears are made as usual.
3. This method is reported to obtain specimens that are more highly cellular but less bloody than those obtained by the aspiration method.

E. Swabs
1. Specimens from ear canals, fistulous tracts, exudates, and vagina or endometrium are obtained by swabbing the tissue with a moistened sterile cotton swab.
2. Smears are prepared by gently rolling the swab on a slide to form a linear preparation.
3. The swab should not be smeared back and forth on the slide because this method causes excessive cellular damage and lysis.

F. Fluids
1. Fluids should be collected into tubes containing EDTA to prevent clotting.
2. If the fluid also will be cultured or used for biochemical tests, a separate aliquot should be collected on a sterile swab or in a sterile tube. The collection tube for microbiological culture should not contain EDTA because it is bacteriostatic.
3. Unless the sample will be processed within an hour or two, smears should be prepared immediately to prevent cell deterioration and post-collection bacterial overgrowth.
4. Direct, blood-type smears can be prepared from cloudy fluids.
5. Squash preparations can be made from any particulate material in fluids.
6. Fluids that appear clear or only slightly turbid are usually sparsely cellular and must be concentrated for smears to be cellular enough to be diagnostic. The fluid can be centrifuged (as for urine sediment preparations), the majority of supernatant decanted, and cells resuspended in a drop or two of the remaining fluid. Blood-type smears are made from the concentrated cell suspension.
7. Fluid specimens requiring cell count and protein determination that are sent to a laboratory for analysis should be submitted as an aliquot of fluid in EDTA along with two or three unstained slides. Although reasonably accurate cell counts and protein concentrations can be obtained from fluid that has been in transit for several days, the cells deteriorate within hours and cellular morphology cannot be evaluated accurately on such old specimens.

G. Smear preparation
1. Cells must be in a monolayer and flattened to be examined cytologically. A variety of methods provides diagnostic smears.
2. Blood-type smears usually provide the most diagnostic preparation. They are useful when the collected material is soft (e.g., lymph node aspirates, soft masses) or a mixture of tissue fluid and

Free ebooks ==> www.Ebook777.com

particulate material. With the tip of the needle touching the slide, the harvested material is gently expressed onto the slide in a single drop. A second slide is brought up to the drop and a smear is made. When a large amount of material is obtained with aspiration and the drop is larger than needed for a smear, multiple smears can be made.

3. Squash preparations are used when the material is too firm or tenacious for blood-type smears.

a. They are prepared by placing a second slide on top of the slide containing the specimen and the two slides are pulled apart, producing two smears. The weight of the slide is sufficient to spread out the material.

b. When excessive pressure is placed on the spreader or upper slide as the two are pulled apart, cells may be lysed. These smears have a tendency to be too thick.

4. Needle smears are prepared by using the syringe needle to pull the aspirated material outward in several different directions to produce a star-shaped smear.

a. This method frequently produces a thick smear with the majority of cells insufficiently flattened for microscopic examination.

b. Cells at the tips of the projections often form an adequate monolayer for microscopic examination.

5. *The material should not be sprayed onto a slide* because this produces droplets that are too thick and the cells are in multiple layers and insufficiently flattened to examine and identify. Thinner droplets usually consist of free nuclei and nuclear debris of lysed cells.

6. Unstained smears must be protected from formalin fumes because they interfere with Romanowsky staining and may render the smears nondiagnostic.

II. MASSES, LESIONS, AND TISSUES (FIGURE 12.1)

The first objective of cytologic evaluation of tissue and mass smears is to differentiate between inflammatory and noninflammatory conditions. Noninflammatory lesions can then be separated into neoplastic and nonneoplastic conditions. Mixtures of inflammatory cells and tissue cells present a diagnostic challenge because it may be difficult cytologically to differentiate a neoplasm with inflammation from inflammation with hyperplasia of a normal cellular component. Some aspirates may not yield diagnostic information, either because of the quality of the smears or because some lesions cannot be diagnosed cytologically. In such instances, histopathology is required for diagnosis.

A. Inflammatory lesions

1. The inflammatory cell type that predominates characterizes the exudate (purulent, pyogranulomatous, granulomatous, eosinophilic, lymphocytic) and may suggest the cause of the lesion.

2. Purulent exudates usually are associated with infectious agents, particularly bacteria, and a diligent search for organisms should be conducted. Depending on the particular organism involved, neutrophils are nondegenerate or exhibit varying degrees of degeneration. Degenerate neutrophils indicate a toxic environment. They have swollen, smooth chromatin that stains less intensely.

3. Macrophages are numerous or predominate in infections caused by fungal organisms, foreign material, protozoa, and mycobacteria. The type of inflammation associated with such conditions is commonly granulomatous or pyogranulomatous.

4. A predominance of eosinophils usually is associated with allergic reactions, parasites, some infectious agents (e.g., *Pythium*), and eosinophilic granulomas.

5. Injection site reactions are associated with a mixed cell population in which small lymphocytes predominate. Macrophages, plasma cells, eosinophils, and neutrophils are less numerous. Frequently, bright pink-purple granular to globular (injected) material may be seen within macrophages as well as free in the background of the smear.

www.Ebook777.com

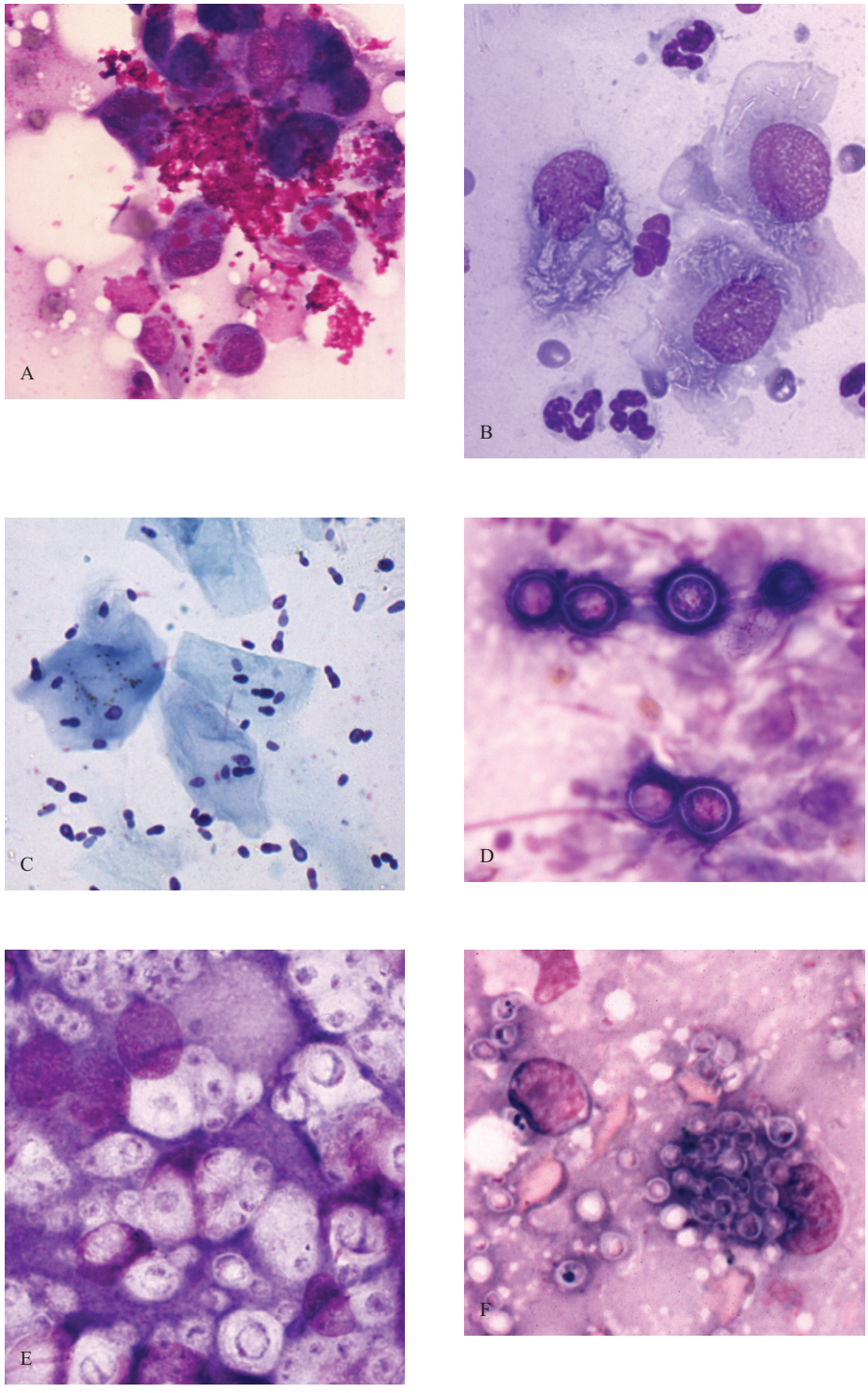
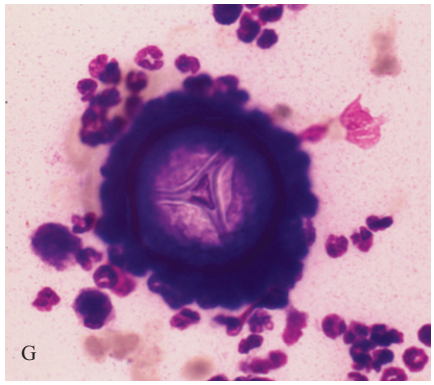
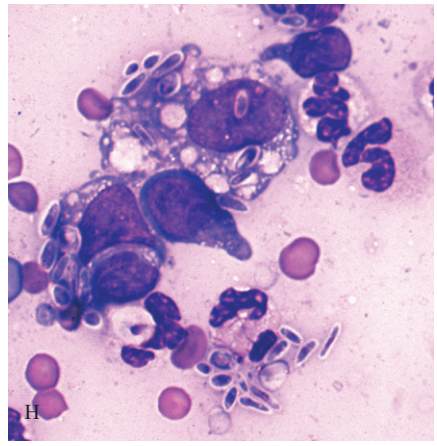
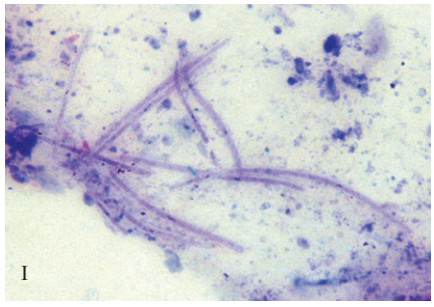


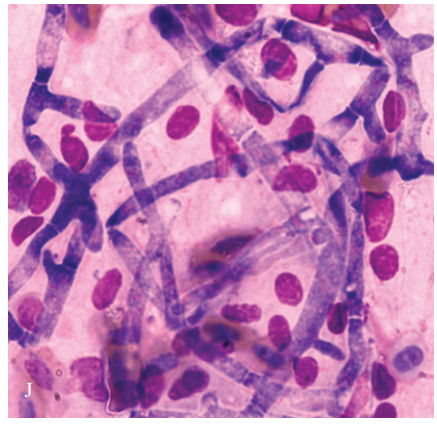
FIGURE 12.1. A. Injection site reaction with intracellular and extracellular globular foreign material. B. Unstained rod-shaped structures consistent with mycobacteria are present within the cytoplasm of macrophages. C. Footprint-shaped *Malassezia* yeasts. D. *Blastomyces dermatitidis* yeasts. E. *Cryptococcus neoformans* yeasts. F. *Histoplasma capsulatum* yeasts within macrophages; continued.

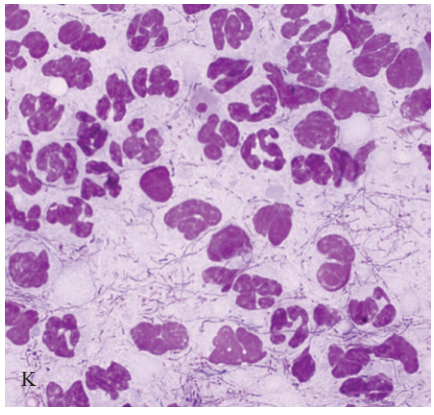
Free ebooks ==> www.Ebook777.com











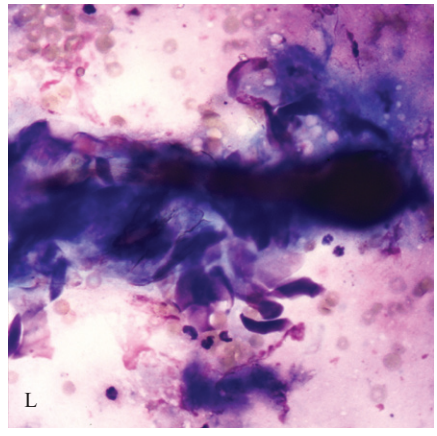
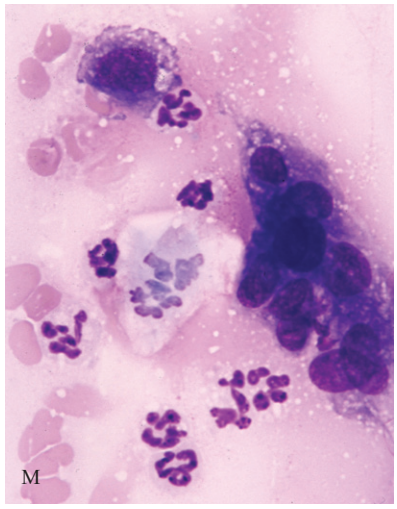
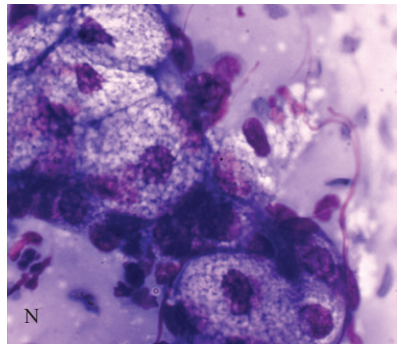
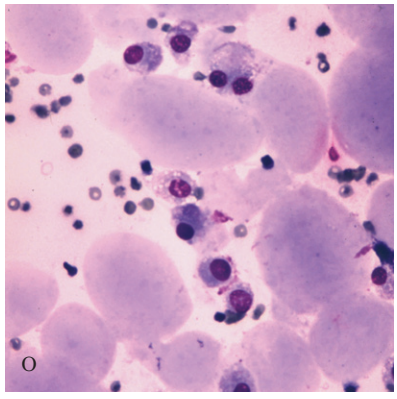


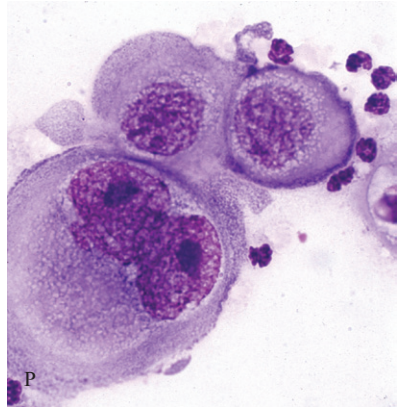
FIGURE 12.1. continued. G. *Coccidioides immitis* yeasts surrounded by inflammatory cells. H. *Sporothrix schenckii* yeasts. I. Filamentous organisms typical of megabacteria of birds. J. Fungal hyphae. K. Filamentous beaded bacteria typical of *Nocardia* sp. or *Actinomyces* sp. and degenerate neutrophils. L. Keratin-producing cyst or tumor; continued.

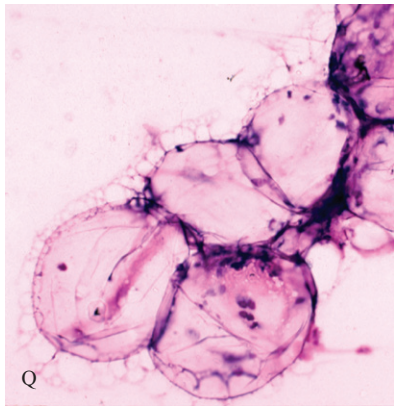
www.Ebook777.com











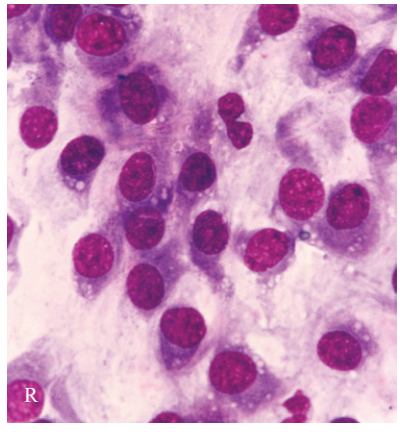
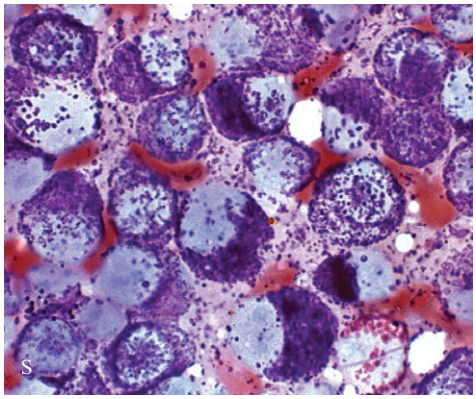
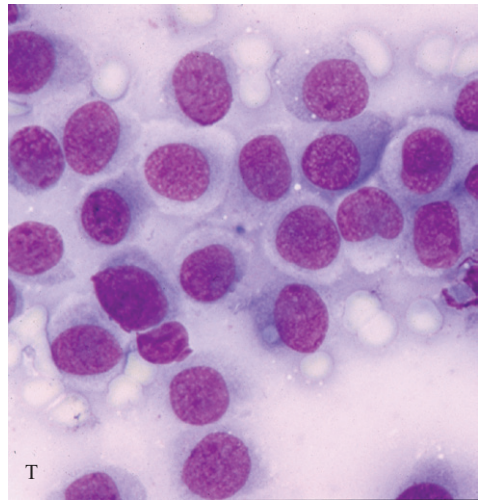
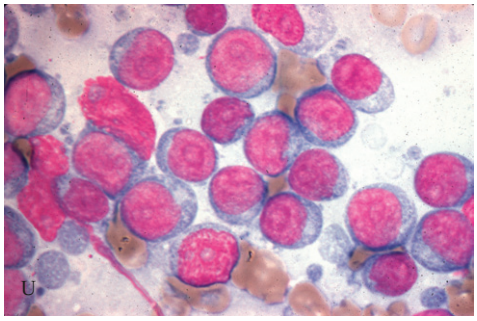


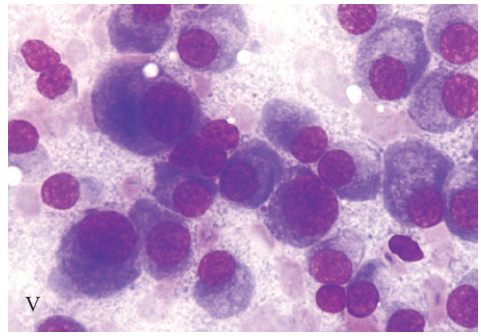
FIGURE 12.1. continued. M. Pyogranulomatous inflammation associated with a ruptured keratin-producing cyst or tumor. N. Sebaceous cells. O. Large amorphous masses of mucin and macrophages from a sialocele. P. Prominent anisocytosis and anisokaryosis in tumor cells from a squamous cell carcinoma. Q. Mature lipocytes from a lipoma or normal fat. R. Plump spindle cells from a hemangiopericytoma; continued.

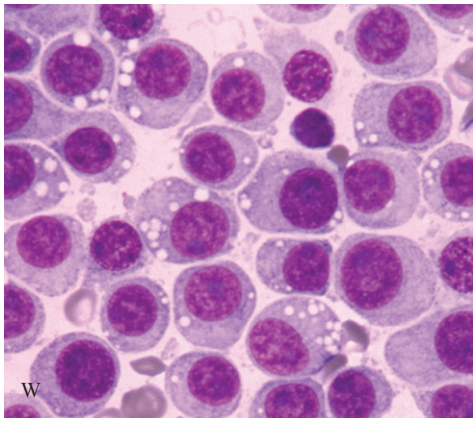
Free ebooks ==> www.Ebook777.com











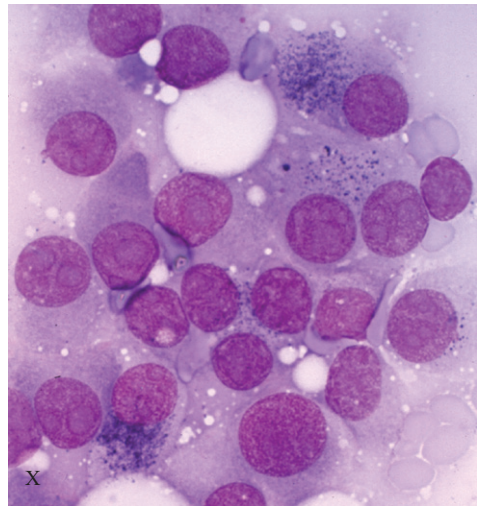


FIGURE 12.1. continued. S. Mast cell tumor. T. Histiocytoma. U. Lymphosarcoma. V. Plasmacytoma. W. Canine transmissable venereal tumor. X. Malignant melanoma.

www.Ebook777.com

6. Xanthomas are common skin lesions of birds such as Parrots, Cockatiels, and Budgerigars that are fed excessive sunflower seeds. Cutaneous xanthomas consist of lipid-laden macrophages, multinucleated giant cells, and occasional cholesterol crystals.

7. A variety of infectious agents can be seen in inflammatory lesions and many can be identified definitively by cytologic examination.

a. Bacteria are the most common organisms observed in cytologic preparations.

(1) With Romanowsky staining, the majority of bacteria appear dark blue as compared to purple-staining nuclei and nuclear debris.

(2) Bacteria may be cocci, bacilli, coccobacilli, or filaments.

(3) *Clostridium* spp. appear as large, blue bacilli with an unstained spore that may give the organism a safety-pin-like appearance. Clostridial enteritis is relatively common in some species of birds such as lorikeets.

(4) Mycobacteria fail to stain with Romanowsky stains and appear as clear, rod-shaped, refractile structures within macrophages or in the background of the smear.

(5) Bacteria usually are extracellular or have been phagocytosed by neutrophils. They are rarely present in macrophages, with the exception of *Mycobacteria* spp. Bacteria phagocytosed by neutrophils may be seen in macrophages when the neutrophil that contains the bacteria is phagocytosed by the macrophage.

(6) Microbiological culture usually is required for definitive identification of most bacteria.

b. Superficial yeasts

(1) *Malassezia* spp. are footprint- or peanut-shaped yeasts commonly seen in cytologic preparations from inflamed ears and skin imprints from dogs and, less frequently, cats.

(2) *Candida* spp. are oval to egg-shaped, basophilic yeasts that may be seen in the oral cavity of many species or in cloacal swabs of birds. They also form hyphae and pseudohyphae.

c. The agents of deep, systemic, and miscellaneous mycoses

(1) *Blastomyces dermatitidis* is a round, basophilic yeast that generally measures 6 to 15 µm in diameter (approximately the size of an erythrocyte to slightly larger than a neutrophil) and has a thick, distinct, clear, refractile capsule. This organism usually stains dark blue and exhibits broad-based budding.

(2) *Cryptococcus neoformans* is a yeast that most commonly causes infections in cats. The organisms range from 2 to 20 µm in diameter and usually have a thick, clear, unstained, mucopolysaccharide capsule. They may be present in large numbers admixed with very few inflammatory cells.

(3) *Histoplasma capsulatum* is smaller than the above-mentioned yeasts, usually measuring 2 to 5 µm in diameter. The organisms are round to slightly oval, pale blue yeasts surrounded by a thin, clear halo. They usually are present within macrophages that typically contain multiple yeasts within their cytoplasm.

(4) *Coccidioides immitis* is the largest of the systemic mycotic agents. It is usually in low numbers in lesions and thus can be missed in cytological preparations. The organisms are 20- to 200-µm diameter spherules with a thick, refractile cell wall and granular basophilic protoplasm or multiple internal endospores that measure 2 to 5 µm in diameter.

(5) *Sporothrix schenckii* may be associated with proliferative to ulcerated skin lesions, most commonly in cats, dogs, and horses. The organisms are round to cigar-shaped, measure 3 to 9 µm long × 1 to 3 µm wide, and stain blue. Organisms are numerous in feline lesions, but they are infrequent to rare in canine lesions.

(6) The so-called megabacteria of birds are now considered fungi that may be observed in cloacal swabs of affected and some clinically healthy birds. These organisms are rod-shaped to filamentous and measure approximately 1 mm in length (approximately the size of a microfilaria). They stain blue with Romanowsky and Gram's stains.

(7) Many fungi that cause infections of various tissues form hyphae.

Free ebooks ==> www.Ebook777.com

(a) The majority of these are not identifiable on the basis of cytologic features and require microbiological culture for identification.

(b) Fungal hyphae are recognized as linear structures, usually measuring in the range of 5 to 10 µm in width, that may have internal septation and/or branching.

(c) The hyphae usually stain blue, but some fungi stain poorly or may not stain at all but may be visible as clear, elongate structures.

(d) Some fungi are pigmented (i.e., dematiaceous fungi) and are various shades of gold, brown, or black.

(e) The *Zygomycetes* and *Pythium* spp. may provoke eosinophilic inflammation.

d. Protozoa are observed infrequently.

(1) Coccidia that infect enterocytes and deep tissues (e.g., *Sarcocystis* spp., *Toxoplasma gondii*) often appear as crescent- or banana-shaped organisms with a small purple nucleus and poorly staining cytoplasm.

(2) *Trichomonas* sp. is observed commonly in caseous, oral lesions of free-ranging birds such as doves and pigeons. In Romanowsky-stained preparations they appear as pear-shaped organisms with a small nucleus, curvilinear nonstaining structure (axostyle), and anterior flagella. They may be recognized readily in cytologic wet mounts by their undulating movement.

(3) *Leishmania* sp. may be associated with cutaneous lesions. These organisms are 2 to 3 µm in diameter and slightly oval, with a small nucleus and kinetoplast that appears as a flat bar.

e. *Prototheca* sp. are classified as achlorophyllous algae. They vary from 15 to 40 µm in diameter and have a clear cell wall. They appear compartmentalized because of endosporulation. Internally, they have a blue to purple, speckled appearance.

B. Noninflammatory, nonneoplastic lesions

1. Keratin-producing lesions

a. Epidermal and follicular cysts contain keratin.

b. These lesions yield a gray or white, pasty material composed of keratin, cornified cells, debris, cholesterol crystals, and occasional squamous epithelial cells.

c. Certain benign keratin-producing tumors (e.g., intracutaneous cornifying epithelioma, pilomatrixoma, trichoepithelioma) also may yield keratin and epithelial cells. These neoplasms may be cytologically indistinguishable from cysts.

d. Keratin-producing cysts and tumors may rupture, and free keratin incites a foreign body inflammatory response. In such cases, pyogranulomatous inflammation comprised of neutrophils, macrophages, and multinucleated giant cells are mixed with the cyst contents.

2. Sebaceous gland hyperplasia is usually a wart-like mass comprised of uniform, large, round cells with moderately abundant cytoplasm that contains many round, clear vacuoles. Nuclei are round and have a single, centrally located nucleolus.

3. Sialocele

a. Grossly, a sialocele appears as a soft, fluctuant, ventral cervical or submandibular mass.

b. Cytologically, the fluid contains large vacuolated macrophages and variable numbers of neutrophils, erythrocytes, and occasional bright gold rhomboidal hematoidin crystals derived from breakdown of hemoglobin.

c. Mucin appears as irregular masses of hyaline, pale blue material, or a more diffuse pink to blue granular to fibrillar background material.

4. Seroma/hematoma

a. The fluid is sparsely cellular and has a high protein content, visible as a basophilic background.

b. Monocyte-macrophages are the principle cell type. Macrophages frequently contain phagocytosed cellular debris within their cytoplasm, particularly erythrocytes. Leukocytes are

www.Ebook777.com

present in low numbers and often appear degenerate. Hemosiderin and/or hematoidin may be seen in older lesions.

c. A finely granular, eosinophilic, proteinic background may be present.

d. Variable numbers of erythrocytes are present. If erythrocytes are present in large numbers, the lesion is probably a hematoma.

C. Neoplasms

1. Neoplasms can be classified as epithelial or mesenchymal/connective tissue tumors. Mesenchymal tumors are further subdivided as spindle cell or round cell tumors.

2. In the case of well differentiated neoplasms, a specific diagnosis may be made by cytologic examination. Less well differentiated tumors, however, may only be identifiable as epithelial or mesenchymal in origin. Some tumors may be insufficiently differentiated for classification at all. When architectural organization of the neoplasm and/or evidence of local invasion must be evaluated for diagnosis or grading, histopathology is needed for definitive diagnosis.

3. Classification of a neoplasm as malignant is based on the presence of morphologic criteria of malignancy, which primarily involve nuclear features (Table 12.1).

4. Epithelial tumors

a. Epithelial tumors usually exfoliate readily, so smears tend to be cellular. The cells frequently are arranged in clumps, clusters, and sheets. Occasional tubular or acinar arrangements may be seen in smears from adenomas and adenocarcinomas.

b. Individual cells are polyhedral and have a round to oval nucleus, fine chromatin pattern, a single nucleolus (usually), and moderately abundant cytoplasm.

c. Squamous cell carcinoma

TABLE 12.1
CYTOMORPHOLOGIC FEATURES OF MALIGNANCY.

General features
Macrocytosis (cell enlargement)
Monomorphic population (belonging to a single cell line)
Pleomorphic
Anisocytosis (variation in cell size)
Poikilocytosis (variation in cell shape)
Nuclear features
Anisokaryosis (variation in nuclear size)
High nuclear:cytoplasmic ratio (nuclear enlargement greater than increase in cytoplasm)
Increased number of mitotic figures, abnormal mitotic figures
Macrokaryosis (nuclear enlargement)
Multinucleation
Nuclear molding (nuclear deformation by adjacent cells)
Variation in number, size, and shape of nucleoli

Free ebooks ==> www.Ebook777.com

(1) The cells frequently are sufficiently characteristic to permit a cytologic diagnosis.

(2) Neoplastic squamous cells have moderate to abundant, hyaline, pale blue cytoplasm with perinuclear vacuolation or pallor.

(3) Purulent inflammation commonly is associated with these neoplasms. It can be difficult to distinguish a squamous cell carcinoma with inflammation from a chronic inflammatory lesion with squamous cell hyperplasia and dysplasia.

5. Spindle cell tumors

a. This group of tumors is derived from connective tissue. Neoplasms are comprised of cells that may be spindloid, stellate, or polyhedral. Nuclei are oval to elongate and the cytoplasm merges gradually with the background of the smear.

b. Smears usually contain few cells because the supporting collagenous stroma surrounding the cells prevents their detachment or exfoliation.

c. This group of neoplasms includes fibroma/fibrosarcoma, lipoma/liposarcoma, hemangiopericytoma, hemangioma/hemangiosarcoma, peripheral nerve sheath tumors, osteoma/osteosarcoma, chondroma/chondrosarcoma, myxoma/myxosarcoma, malignant fibrous histiocytoma, leiomyoma/leiomyosarcoma, and rhabdomyoma/rhabdomyosarcoma. These neoplasms usually are difficult to differentiate from each other cytologically. Specific diagnosis and frequently determination of malignancy require histopathology.

d. Hyperplastic connective tissue (i.e., granulation tissue) cytologically resembles connective tissue tumors. Histopathology usually is required to distinguish hyperplasia from neoplasia.

e. Lipoma

(1) Lipomas are comprised of well differentiated lipocytes (adipocytes) and are indistinguishable from normal fat.

(2) Smears consist of oily appearing, nonstaining, lipid droplets, and large, clear round cells with a small, pyknotic, peripherally displaced nucleus.

(3) Lipomas are common neoplasms of dogs and certain birds such as Budgerigars, Cockatiels, and some species of parrots. They occur rarely in cats.

f. Hemangiopericytoma

(1) Unlike the majority of spindle cell tumors, hemangiopericytomas frequently exfoliate readily, producing moderately cellular smears. These cells frequently are sufficiently characteristic to allow a definitive cytologic diagnosis.

(2) Individual cells are stellate or spindloid and have a round to oval nucleus, fine chromatin pattern, and one or two nucleoli that are usually small. The cytoplasm is pale to medium blue, may have small vacuoles, and sometimes resembles a veil around the nucleus. Binucleate and multinucleate cells are commonly present.

(3) These cells are frequently in loose sheets or clumps in the smear and occasionally are arranged in whorls that are characteristic of this tumor.

6. Round cell tumors

a. This group of neoplasms includes various mesenchymal tumors comprised of discrete round to polyhedral cells. Cells generally exfoliate in large numbers. Diagnosis is based solely on cellular appearance because architectural organization of the cells is not an important diagnostic criterion. Therefore, cytology is frequently better than histopathology for diagnosis of round cell tumors because cellular morphology is more clearly visible in smears than in tissue sections.

b. Mast cell tumor

(1) Mast cells have moderate amounts of cytoplasm that contains variable numbers of metachromatic (purple) granules. Nuclei stain poorly when cells are highly granulated. Granules may not stain or stain very poorly with Diff-Quik® stain (Dade International, Inc.).

(2) Variable numbers of eosinophils, macrophages, and connective tissue spindle cells also may be present.

(3) Free metachromatic granules from ruptured cells and mucin are present in the background.

www.Ebook777.com

(4) Poorly differentiated tumors exhibit anisocytosis and anisokaryosis, and are more sparsely granulated; however, grading is based on histologic criteria of tissue sections.

c. Histiocytoma

(1) This is a tumor of epidermal dendritic (Langerhans) cells that frequently undergoes spontaneous regression.

(2) This tumor is relatively unique to dogs.

(3) The cells have oval, reniform, or folded nuclei with fine chromatin and inconspicuous nucleoli. The cytoplasm is moderate in amount and appears pale blue.

(4) Fewer cells are usually aspirated in comparison to the other types of round cell tumors.

d. Lymphoma (lymphosarcoma)

(1) This tumor is comprised of a uniform population of immature lymphocytes that are medium- (approximately size of neutrophils) to large-sized cells.

(2) The neoplastic cells have a round, oval, or polyhedral nucleus with a fine chromatin pattern and multiple and/or large nucleoli. The cytoplasm is scant to moderate and dark blue, and may infrequently contain fine vacuoles or dust-like azurophilic (purple) granules.

(3) Cytoplasmic droplets may be numerous in the background of the smear, particularly when the cells are very large.

e. Plasmacytoma

(1) The neoplastic cells have a round, eccentrically situated nucleus with a small to moderate amount of medium blue cytoplasm. A paranuclear patch of cytoplasmic pallor represents the Golgi zone that contains immunoglobulin.

(2) Binucleate and multinucleate cells usually are observed.

(3) Anisokaryosis and anisocytosis may be prominent.

f. Transmissible venereal tumor

(1) This round cell tumor is unique to dogs.

(2) The cells comprising this tumor have a round nucleus with coarse, cord-like chromatin and a prominent, large nucleolus. The cytoplasm is moderate in amount and medium blue with several clear, round vacuoles. Cellular margins are distinct.

(3) Mitotic figures are frequent.

(4) Lymphocytes, plasma cells, and macrophages may be scattered among the tumor cells.

(5) The tumor is usually located on the genitalia but occasionally occurs in the nasal cavity and skin (secondary to bite wounds).

g. Melanoma

(1) Neoplastic cells may be round, polyhedral, or spindle-shaped and usually exfoliate individually, so this tumor is frequently included in the group of round cell tumors.

(2) The cytoplasm contains scant to abundant melanin pigment that varies from fine, dust-like to globular particles and appears black-brown to green-black.

(3) The nucleus of neoplastic melanocytes is round to oval and usually has a single, large, centrally located nucleolus.

III. LYMPH NODES

Indications for aspiration include any unexplained enlargement of lymph node(s) and to detect metastasis of a tumor to draining lymph nodes (Figure 12.2).

A. Normal lymph node

1. Normal lymph nodes are comprised primarily of small lymphocytes (75% to 95%). They have round nuclei with dense chromatin and a thin rim of cytoplasm. They are slightly larger than erythrocytes. Lymphocytes are fragile and easily ruptured. Thus, free nuclei of lysed cells are common in lymph node smears and must not be confused with large lymphocytes.

Free ebooks ==> www.Ebook777.com

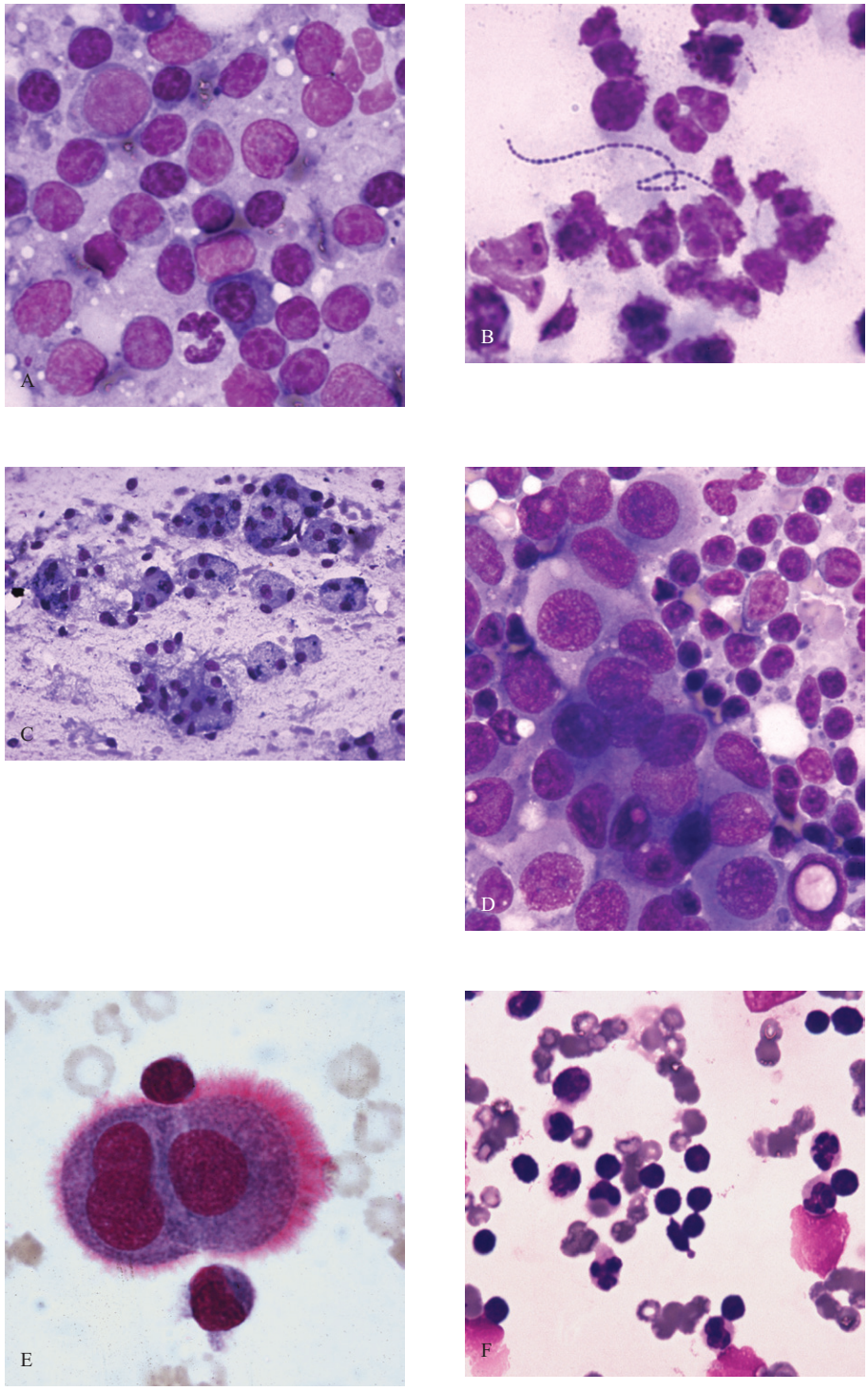


FIGURE 12.2. (A—D) Lymph node aspirates: A. Normal lymph node with small lymphocytes predominating several free nuclei and one plasma cell. B. Degenerate neutrophils and chains of bacterial cocci from *S. equi* lymphadenitis. C. Normal salivary gland cells. D. Metastatic mammary carcinoma cells in lymph node. (E—I) Body cavity effusions: E. Mesothielial cells. F. Small lymphocytes in chylothorax; continued.

www.Ebook777.com

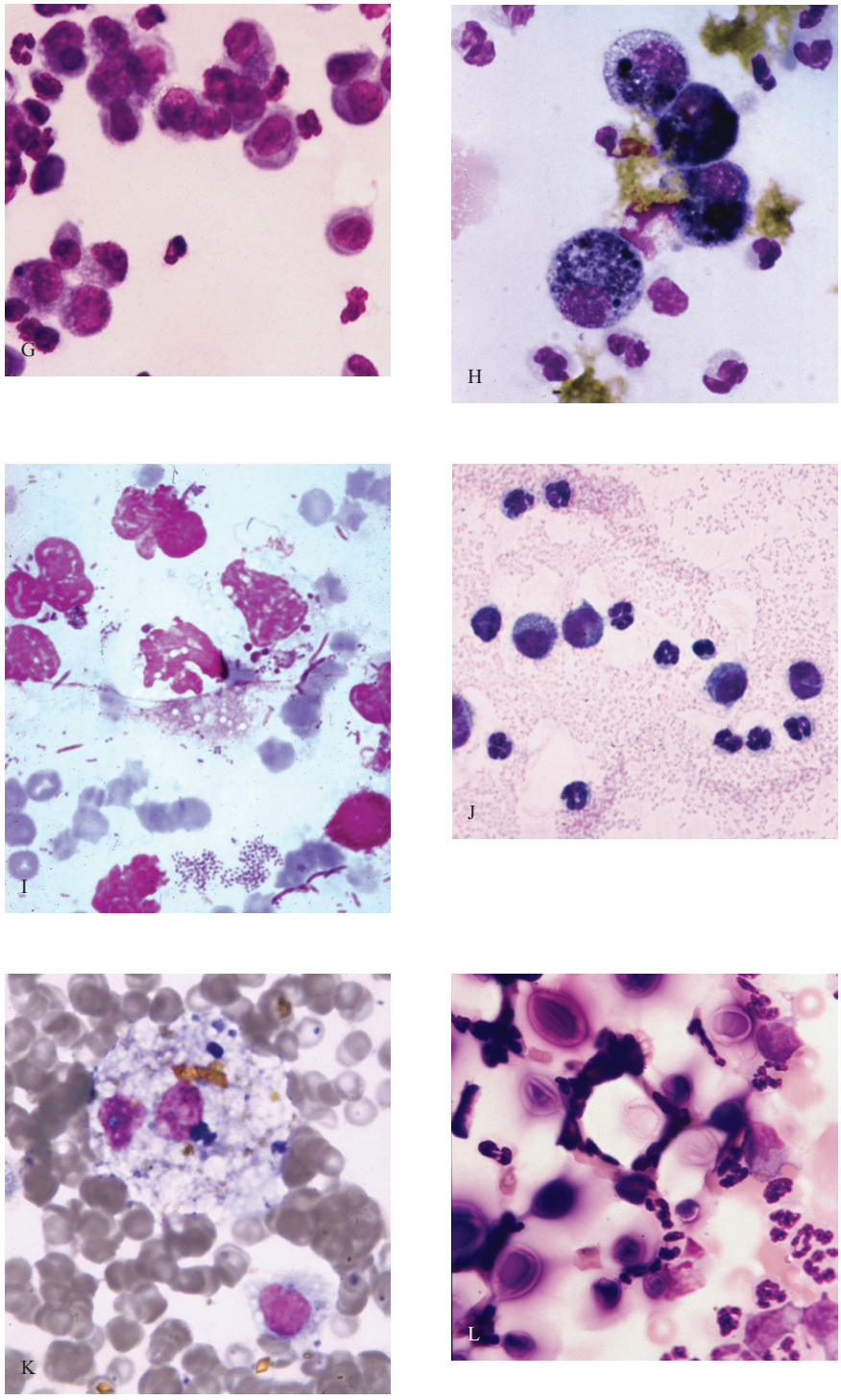


FIGURE 12.2. continued. G. Macrophages, neutrophils, and a diffuse pink background precipitate from abdominal effusion caused by feline infectious peritonitis. H. Intracellular and free bile pigment in bile peritonitis. I. Degenerate neutrophils and mixed bacteria from pyothorax. (J—K) Joint fluid: J. Neutrophilic inflammation. K. Macrophage containing hemosiderin and hematoidin crystals due to chronic hemorrhage. L. Cerebrospinal fluid with pleocytosis and *Cryptococcus neoformans* yeasts; continued.

Free ebooks ==> www.Ebook777.com

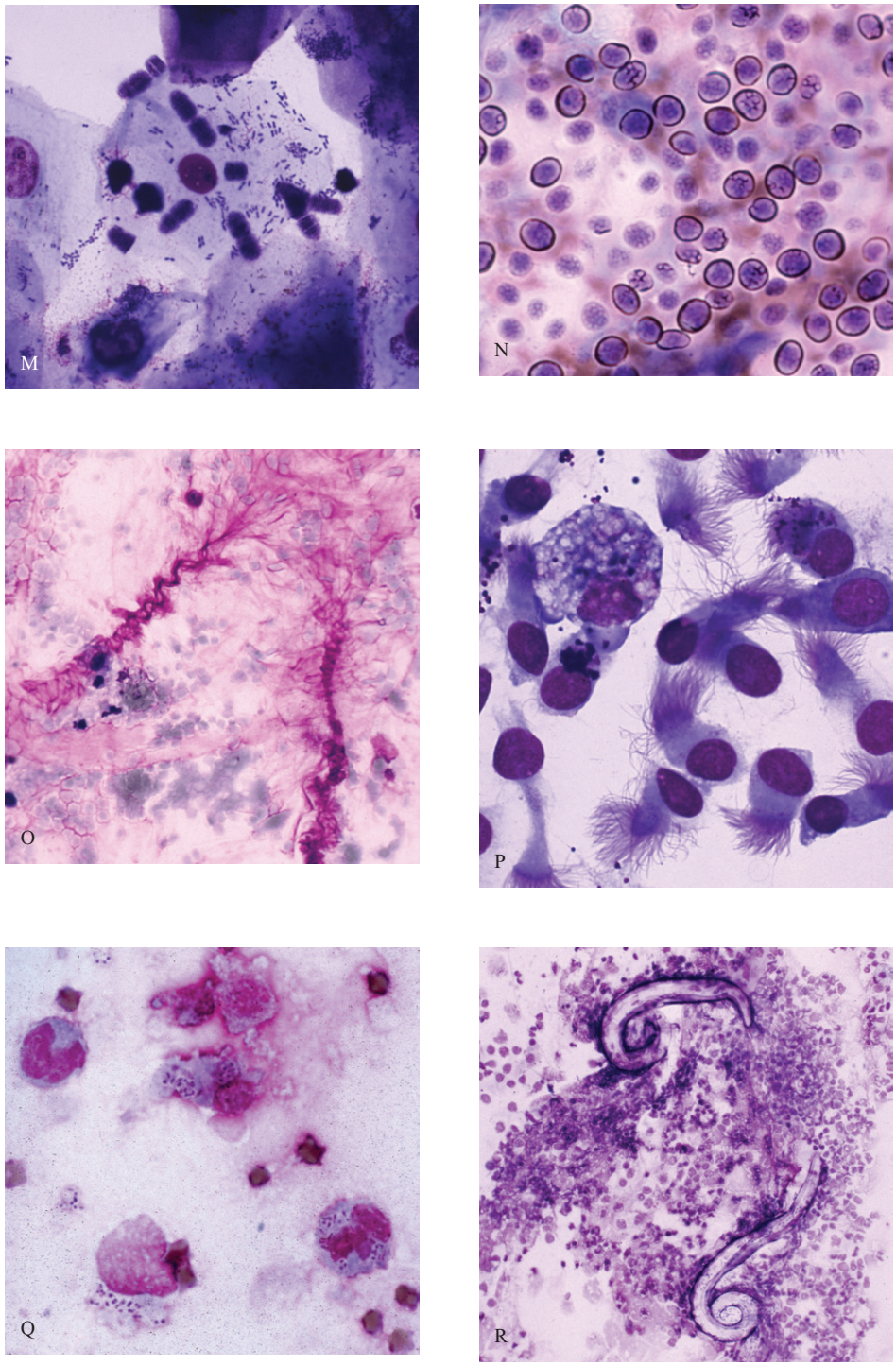


FIGURE 12.2. continued. M. Squamous cells with adherent *Simonsiella* sp. and other bacteria from oral cavity. N. *Rhinosporidium seeberi* organisms from nasal cavity. (O—T) Tracheoalveolar washes: O. Curschmann's spirals. P. Ciliated epithelial cells and alveolar macrophages. Q. *Rhodococcus equi* and degenerate neutrophils. R. Two *Aelurostrongylus abstrusus* larvae and eosinophils;continued.

www.Ebook777.com

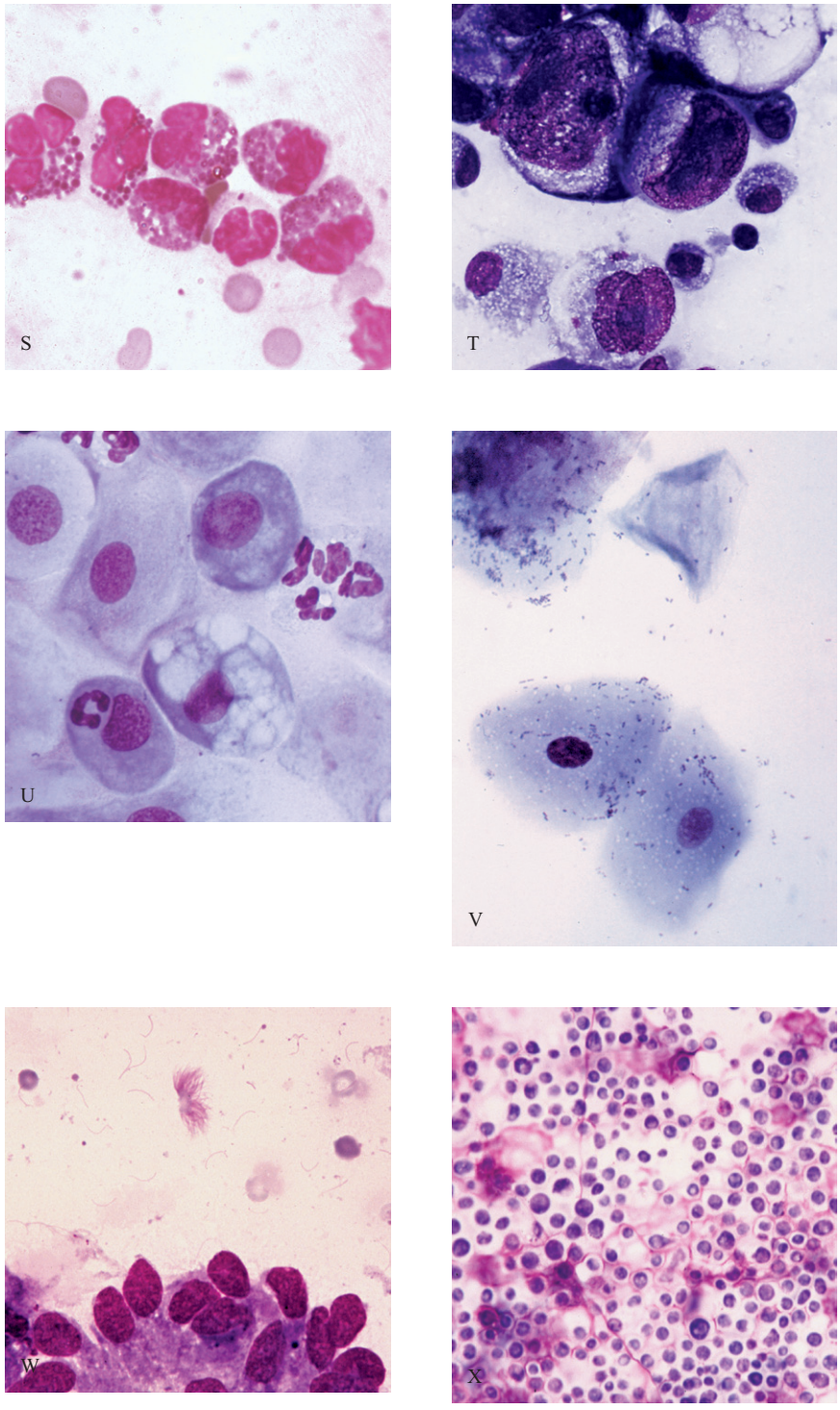


FIGURE 12.2. continued. S. Eosinophils. T. Pulmonary carcinoma. (U—V) Vaginal cytology: U. Noncornified epithelial cells and neutrophils during proestrus. V. Cornified/superficial cells predominant during estrus. (W—X) Endometrial cytology: W. Endometrial epithelial cells and free cilia. X. *Candida* sp. yeasts.

Free ebooks ==> www.Ebook777.com

2. Small numbers of plasma cells, medium- (approximately size of neutrophils) and large-sized lymphocytes, macrophages, granulocytes, and occasional mast cells usually are present.

B. Hyperplastic/reactive lymph node (Case 9)

1. Lymph node hyperplasia can be focal or generalized and is caused by stimulation of the immune system by antigens arriving via afferent lymphatics.

2. The cytologic appearance of a hyperplastic lymph node is similar to that of a normal lymph node but the node is clinically enlarged. Medium- and large-sized lymphocytes and plasma cells may be slightly more numerous than in normal lymph node aspirates.

C. Lymphadenitis

1. Depending on the etiology, inflammation may be primarily neutrophilic, eosinophilic, granulomatous, or a combination of cell types.

2. Neutrophils may be degenerate or nondegenerate and usually are associated with bacterial infections.

3. Eosinophils usually are associated with allergic or parasitic reactions. The disease process often is distant to the lymph node (e.g., dermatitis, enteritis, pneumonitis).

4. Granulomatous reactions are comprised of epithelioid macrophages and, frequently, multinucleated giant cells. Granulomatous inflammation is associated most often with fungal, protozoal, or mycobacterial infections.

D. Lymphoma (Case 10)

1. Normal cells are replaced by a uniform monomorphic population of usually immature, medium- to large-sized lymphoblasts. These cells are the size of neutrophils or larger and have a fine chromatin pattern; prominent nucleoli; and moderately abundant, dark blue, granular cytoplasm.

2. When 70–80% or more of the cells are medium- or large-sized lymphocytes, the diagnosis of lymphoma can be made cytologically. Early stages of lymphoma may be missed, but if multiple enlarged lymph nodes are aspirated, the diagnosis usually can be made.

3. The rare form of small cell lymphoma may be very difficult to differentiate from hyperplasia cytologically.

E. Metastatic neoplasia

1. The presence of cells not normally observed in lymph node aspirates (e.g., any epithelial cells) or increased numbers of cells that are present normally in only small numbers (e.g., mast cells) indicate metastatic neoplasia.

2. Epithelioid macrophages must be differentiated from epithelial cells. Normal salivary epithelial cells, which are frequently obtained when submandibular masses are aspirated, must be recognized and not confused with metastatic epithelial cells.

IV. BODY CAVITY EFFUSIONS

Effusions are classified as transudates, modified transudates, and exudates based on the protein concentration and nucleated cell count. Although all effusions don't easily fit these categories and there is some overlap between the groups, these cytologic classifications are helpful in limiting the potential causes for a particular case of effusion.

A. General considerations

1. Fluid cannot be obtained from the body cavities of small animals unless there is an effusion. In large animals, however, fluid usually can be aspirated from the peritoneal cavity and frequently from the thorax in the absence of disease. Birds lack a diaphragm and have a single coelomic cavity instead of distinct thoracic and peritoneal cavities.

2. Evaluation of a body cavity effusion can help determine the cause of the disease process or provide information to suggest additional diagnostic tests to determine a specific diagnosis.

www.Ebook777.com

3. A variety of methods is available for fluid collection; and once obtained, the fluid should be placed in a tube containing EDTA to prevent clotting. If fluid will be cultured, an aliquot should be placed in a sterile tube without EDTA, which is bacteriostatic and can interfere with culture results.
4. If a delay of more than one or two hours is anticipated before the sample is processed, smears should be prepared immediately to prevent autolytic changes that interfere with cytologic examination, bacterial overgrowth, and *in vitro* changes (e.g., phagocytosis of erythrocytes by macrophages, degeneration of cells) that confuse cytologic interpretation. Fluid that is clear or only slightly turbid likely has low cellularity. Smears of these specimens should be made from centrifuged sediment to concentrate cells and other material to optimize the chances of making a diagnosis.

B. Fluid analysis
1. Color
   a. Normal fluid is clear or slightly opalescent and colorless to pale yellow.
   b. Red color is due to iatrogenic or real hemorrhage. Aspirating the spleen may yield a cytologic sample that resembles peripheral blood.
   c. Turbidity is caused by increased cellularity, bacteria, fibrin, lipid, or ingesta from gastrointestinal rupture or accidental enterocentesis.
   d. Dark green color is caused by bile. Yellow discoloration is caused by urine.
2. Protein
   a. Protein determination can be done by refractometry or by biochemical methods. Turbidity can interfere with refractometer readings; refractometry is done on the centrifuged supernatant when the fluid is cloudy.
   b. Protein concentration of normal peritoneal fluid is less than 2.5 g/dL.
   c. Transudates have a protein concentration less than 2.5 g/dL.
3. Nucleated cell counts
   a. Nucleated cell counts can be done manually, as for white blood cell counts, or by electronic particle counters.
   b. Values may be in error because cell clumping, cell fragmentation, and non-cellular particulate debris are common in effusions.
   c. Normal peritoneal cell counts in large animals are usually fewer than 5,000 cells/μL; counts between 5,000 and 10,000/μL may or may not be normal; cell counts above 10,000/μL indicate inflammation.
4. Cytology
   a. Direct smears can be used to estimate cellularity when a cell count cannot be done or can be used to verify the accuracy of the measured cell count.
   b. Cell populations in effusions
      (1) Mesothelial cells
         (a) Mesothelial cells line the pleural, pericardial, and peritoneal cavities and visceral surfaces. They readily exfoliate in effusions.
         (b) Mesothelial cells commonly undergo hypertrophy and hyperplasia when effusions develop. They may exhibit anisocytosis and anisokaryosis. Activated mesothelial cells are phagocytic and may be difficult to differentiate from macrophages.
         (c) Mesothelial cells have a round or oval nucleus, fine chromatin pattern, and a single nucleolus. Binucleate and multinucleated cells are common. The cytoplasm is dark blue, and a red-pink fringe frequently outlines the cell. Mitotic figures may be seen.
         (d) The cells are distributed singly and in clusters.
         (e) With inflammation, mesothelial cells become markedly hyperplastic, which produces increased cellular pleomorphism. As mesothelial cells become reactive, anisocytosis, anisokaryosis, binucleation and multinucleation, mitotic figures, and cytoplasmic basophilia become more prominent. Hyperplastic mesothelial cells may be difficult to

Free ebooks ==> www.Ebook777.com

differentiate from neoplastic mesothelial cells (mesothelioma) or malignant epithelial cells (carcinoma).

(2) Macrophages

(a) Macrophages are large cells that originate from blood monocytes. They are a normal component of peritoneal fluid.

(b) Macrophages have an oval to reniform (kidney-shaped) nucleus; fine chromatin pattern; small nucleoli; and moderate to abundant, vacuolated cytoplasm that frequently contains phagocytosed cell debris, erythrocytes, foreign material, or fungal organisms. Macrophages usually do not phagocytose bacteria.

(c) Macrophages may be difficult to differentiate from activated mesothelial cells, but the distinction usually is not important.

(3) Neutrophils

(a) Neutrophils are present in normal peritoneal fluid and are seen in most effusions.

(b) Neutrophils are the predominant cell type in most exudates.

(c) Neutrophils are nondegenerate in fluids with a low degree of toxicity such as transudates and modified transudates. They resemble neutrophils in peripheral blood smears and have nuclear lobes comprised of densely aggregated, darkly staining chromatin.

(d) Degenerate neutrophils indicate a toxic environment and are associated with septic inflammation. They are characterized by swollen nuclear lobes with loose, paler staining chromatin. When neutrophils are degenerate, a search for bacteria and/or culture of the effusion should be done. Autolysis of neutrophils causes similar morphologic changes.

(e) Nuclear hypersegmentation and pyknosis are aging changes of neutrophils that do not indicate toxicity.

(4) Lymphocytes, plasma cells, and mast cells are observed less commonly in effusions.

(5) Eosinophils are usually in low numbers in effusions but they may comprise 60% or fewer of the cells in normal peritoneal fluid of cattle. Effusions containing numerous eosinophils have been associated with various parasitic conditions (e.g., heartworm disease, aelurostrongylosis, *Dioctyphyma renale* infection), presumed hypersensitivity reactions, various neoplasms (e.g., lymphoma, mast cell tumor), and pneumothorax.

C. Classification of effusions

1. Transudate (Case 19)

a. Transudates develop most commonly as a result of decreased plasma osmotic pressure because of hypoalbuminemia. Effusions usually do not develop until the albumin value is less than 1 g/dL (or less than or equal to 1.5 g/dL if hypertension is also present).

b. Uncommon causes of transudative effusions are obstruction of low-protein-containing lymphatics of the intestine and effusion associated with early urinary bladder rupture.

c. The fluid is clear and colorless and has a low protein concentration (less than 2.5 g/dL) and low nucleated cell count (less than 1,500 cells/μL).

d. It contains a mixed population of macrophages, nondegenerate neutrophils, and mesothelial cells.

2. Modified transudate (Case 27)

a. This category has the least specificity because many diseases can be associated with production of a modified transudate.

b. The effusion develops in response to increased hydrostatic pressure or permeability of capillaries and/or lymphatic vessels.

c. The fluid has low to moderate cellularity (1,000 to 7,000 cells/μL) and a variable protein concentration (2.5 to 5 g/dL).

d. Common causes of modified transudates include cardiac disease, neoplasms, hepatic disease, and feline infectious peritonitis (Case 7).

e. Color and turbidity are variable and depend on the cause of the effusion.

www.Ebook777.com

f. Cells typically present in modified transudates include nondegenerate neutrophils, macrophages, reactive mesothelial cells, small lymphocytes, and erythrocytes. If neoplastic cells are seen, a definitive diagnosis can be made.

3. Exudate

a. Exudates develop because of increased vascular permeability caused by inflammation.

b. The fluid varies in color but is usually turbid because of high cell count.

c. The nucleated cell count is usually greater than 5,000 to 7,000 cells/μL.

d. Neutrophils are usually the predominant cells and they may be nondegenerate or degenerate depending on the degree of toxicity of the fluid. Numbers of macrophages, lymphocytes, reactive mesothelial cells, plasma cells, eosinophils, and erythrocytes vary according to the type and severity of the irritant and duration of the disease process.

e. Nonseptic exudates are caused by irritants such as bile, urine, and pancreatic enzymes or because of tissue necrosis (e.g., neoplasms, lung lobe torsion). Neutrophils in such effusions are nondegenerate.

f. Septic exudates are caused by microorganisms which may or may not be evident cytologically. Some bacteria produce toxins that cause neutrophilic degeneration but many organisms do not. Therefore, any exudate should be examined carefully for the presence of infectious agents and/or be cultured.

D. Specific conditions

1. Chylous effusions

a. Chylous effusions are milky white to creamy pink fluid that usually develops in the thorax (chylothorax) but may also occur in the abdomen.

b. The opacity of the fluid is due to the high fat content (in the form of chylomicrons) of lymph fluid being transported from the mesenteric lymphatics to the venous system via the thoracic duct. Lymphocytes entering peripheral blood are also returned to circulation via the thoracic duct.

c. Chylous effusions develop because of trauma to or obstruction of the duct and have been associated with neoplasms, cardiac disease, mediastinal granulomas, dirofilariasis, diaphragmatic hernia, and lung lobe torsion. Some cases of chylous effusion are termed idiopathic because the cause cannot be identified. The condition occurs most commonly as pleural effusion in cats with cardiac disease.

d. The fluid may be a modified transudate or exudate. It is classically comprised almost entirely of small lymphocytes. However, neutrophils and macrophages may predominate in chronic chylothorax or after repeated thoracocentesis.

e. When the diagnosis of chylous effusion is in question, it can be confirmed by comparing thoracic fluid cholesterol and triglyceride values to those of blood. Chylous effusions have a higher triglyceride concentration and lower cholesterol concentration than does the blood. In addition, the cholesterol concentration of the fluid divided by the triglyceride concentration is less than 1 in chylous effusions.

2. Feline infectious peritonitis

a. Effusions caused by feline infectious peritonitis are usually yellow and may contain fibrin clots.

b. The fluid may be a modified transudate or exudate based on the nucleated cell count, but the protein concentration is usually high (greater than 3.5 g/dL).

c. The fluid is a mixture of nondegenerate neutrophils, macrophages, and lymphocytes. A characteristic granular, eosinophilic, proteinic precipitate is evident in the background.

d. The presumptive diagnosis can be confirmed by demonstration of coronaviral antigen within the fluid (e.g., by fluorescent antibody testing).

3. Heart failure

a. Cats with heart failure usually develop chylothorax.

Free ebooks ==> www.Ebook777.com

b. Dogs with congestive heart failure typically develop ascites, which is produced by leakage of high-protein hepatic lymph secondary to increased intrahepatic hydrostatic pressure.
c. Ascitic fluid is a modified transudate containing erythrocytes, macrophages, nondegenerate neutrophils, reactive mesothelial cells, and lymphocytes.

4. Hemorrhagic effusions
a. Hemorrhagic effusions are caused by many conditions but are most commonly due to trauma and neoplasia.
b. A true hemorrhagic effusion must be differentiated from blood contamination and inadvertent splenic aspiration. Hemorrhagic effusions more than a few hours old contain macrophages that have phagocytosed erythrocytes within their cytoplasm. If hemorrhage occurred more than a day or two previously, macrophages contain hemosiderin and/or hematoidin crystals within their cytoplasm. These are pigments derived from the breakdown of erythrocytes.
c. If blood is present in an effusion because of contamination or acute hemorrhage (less than one hour), platelets are visible in smears, whereas erythrophagocytosis is not evident. However, if there is a delay of several hours or more before the fluid is processed, platelets disintegrate and will not be visible and *in vitro* erythrophagocytosis can occur, leading to erroneous cytologic interpretation.
d. Tumor cells are found infrequently in smears of effusions caused by neoplasms, particularly when the PCV is greater than or equal to 20%.
e. Pericardial effusions in dogs are most commonly hemorrhagic and are usually caused by neoplasia (e.g., hemangiosarcoma, heart base tumors, mesothelioma) or are idiopathic (idiopathic/benign pericardial effusion). The diagnosis frequently cannot be made cytologically and histopathology is required.

5. Neoplasia
a. Neoplasms commonly cause effusions in the thorax, abdomen, and pericardial sac, but frequently cannot be diagnosed cytologically because tumor cells may not be exfoliated into the effusion.
b. Cytologic examination is most successful for detecting discrete cell tumors such as lymphoma and mast cell tumors.
c. As a continually proliferating cell population, mesothelial cells typically exhibit some degree of cellular pleomorphism. However, when they are stimulated to proliferate more rapidly, cellular atypia becomes more exaggerated and differentiation of neoplastic from markedly reactive mesothelial cells or reactive mesothelial cells from a carcinoma may not be possible.
d. Carcinomas and adenocarcinomas that seed the surfaces of body cavities exfoliate cells more readily than do most sarcomas.

6. Uroperitoneum
a. Rupture of the urinary bladder, urethra, ureter, or kidney leads to a yellow-tinged effusion that may be a modified transudate or an exudate.
b. The fluid commonly is comprised of a mixture of nondegenerate neutrophils, macrophages, and reactive mesothelial cells.
c. The diagnosis can be confirmed by measuring creatinine concentrations in the effusion and blood. The creatinine concentration of the fluid should exceed that of blood.

7. Bile peritonitis
a. Rupture of the gallbladder or bile duct causes peritonitis characterized by a yellow-green effusion.
b. The fluid is comprised of neutrophils, macrophages, reactive mesothelial cells, erythrocytes, and lymphocytes. Macrophages contain yellow-green to blue-green bile pigment within their cytoplasm.
c. The diagnosis can be confirmed by demonstrating a higher concentration of bilirubin in the fluid than in blood.

8. Egg yolk peritonitis (serositis, coelomitis) in birds
a. This condition may occur in older hens or hens with intense egg production.

www.Ebook777.com

b. Free egg yolk material within the coelomic cavity incites mild to moderate inflammation.
c. Heterophils and macrophages usually are the predominant inflammatory cells.
d. The background of the smear may have a variable appearance. Yolk may appear light pink to blue with a smooth to granular to globular protein precipitate (that occasionally may be reminiscent of FIP in cats).

V. SYNOVIAL FLUID

Synovial fluid analysis, in conjunction with history, physical examination findings, radiography, and other ancillary tests such as culture and serology, is important in the diagnosis of joint diseases. Complete synovial fluid analysis consists of description of the appearance of the fluid, mucin clot test, protein determination, nucleated cell count, and cytologic examination. When the sample volume is limited, cytologic examination is the single most useful test.

A. Appearance
1. Normal synovial fluid is transparent and colorless to pale yellow.
2. Normal synovial fluid does not clot because it lacks fibrinogen and other clotting factors.
3. When synovial fluid is bloody, hemarthrosis must be distinguished from iatrogenic hemorrhage caused by arthrocentesis (more common) (Case 25).
a. Usually this distinction is made at the time of specimen collection.
b. With hemarthrosis, the fluid is uniformly bloody.
c. With iatrogenic hemorrhage, the synovial fluid and blood appear separately and blood may not be present throughout the sample collection.
d. Bloody fluids should be placed into EDTA tubes to prevent coagulation.
4. Chronic hemorrhage is characterized by yellow-orange discoloration, termed xanthochromia, due to the presence of hemoglobin breakdown pigments.
5. Synovial fluid may exhibit thixotrophy. This property is characterized by formation of a gel when the fluid remains undisturbed for several hours and it returns to a fluid state when gently shaken.
6. Turbidity usually is caused by increased cellularity due to inflammation.

B. Viscosity
1. The normal viscosity of synovial fluid is produced by high hyaluronic acid concentration.
2. Viscosity usually is evaluated subjectively by observing the length of strand formed when synovial fluid is expressed from a syringe through a needle. Normal fluid usually forms a strand equal to or greater than 2 cm in length before it breaks.
3. Viscosity is recorded as normal, decreased, or markedly decreased.
4. Viscosity also can be evaluated qualitatively during cytologic examination. In normal synovial fluid smears, cells are arranged in conspicuous rows ('rowing') because of the viscosity of the fluid. As viscosity decreases, the cells become progressively more randomly distributed.
5. Viscosity is decreased because of dilution of hyaluronic acid by effusion into the joint (e.g., inflammation, hydrarthrosis) or by degradation of hyaluronic acid by bacterial hyaluronidase.

C. Mucin clot test
1. This is a semiquantitative test of the quality and quantity of synovial fluid mucin (hyaluronic acid).
2. The test consists of adding 1 part synovial fluid supernatant to 4 parts 2.5% glacial acetic acid. The acid denatures and agglutinates the mucin. The quality of the mucin clot is graded as follows:
a. The test is considered good/normal when a tight, ropey clot forms and the solution is clear.
b. A fair/slightly decreased clot test is when a soft clot forms and the solution is slightly cloudy.
c. A poor clot test consists of a friable clot in a cloudy solution.

Free ebooks ==> www.Ebook777.com

d. The test is considered very poor if a clot does not form and flecks are present in a very cloudy solution.

3. EDTA degrades hyaluronic acid and thus the mucin clot test should not be performed on synovial fluid samples in EDTA.

D. Protein

1. Synovial fluid protein concentration usually is measured by refractometry but can also be measured by biochemical assays.

2. Joint trauma and inflammatory conditions increase the protein concentration.

E. Nucleated cell count

1. Only nucleated cells are counted. Counts can be done manually or by electronic particle counters. Manual counts use a hemocytometer that is loaded directly with synovial fluid or with diluted synovial fluid. Acetic acid cannot be used as the diluent because it causes agglutination. Electronic particle counters dilute the specimen in isotonic buffer.

2. Counts are usually less than or equal to 500 cells/µl in healthy animals but they may be as high as 3,000 cells/µl in the dog.

F. Cytologic examination

1. This is the most important portion of the synovial fluid analysis. If only a drop or two of synovial fluid is available for testing, it should be used to make a smear.

2. Initial examination of the smear should include an evaluation of cellularity (as normal or mildly, moderately, or markedly increased), presence or absence of cell rowing as an indication of viscosity, and number of erythrocytes.

3. Normal synovial fluid consists of large mononuclear cells, which are primarily macrophages, and fewer small mononuclear cells or lymphocytes. Neutrophils usually constitute less than 10% of the nucleated cells. Eosinophils are not present in health.

4. Synovial fluid reactions are usually classified as noninflammatory/degenerative, inflammatory, or acute hemorrhage.

5. In degenerative arthropathies (degenerative joint disease), the volume of synovial fluid varies from normal to markedly increased and the cell count is normal to mildly increased (usually less than 5,000 cells/µl).

a. Cells are predominantly mononuclear. Neutrophils may be slightly more numerous.

b. Protein concentration is within the reference interval or slightly increased and the fluid does not clot.

c. The mucin clot test is usually good unless a large volume of effusion is present.

6. Inflammatory arthropathies may be infectious or noninfectious.

a. The cell count is greater than 10,000 cells/µl (and sometimes up to 100,000 cells/µl).

b. The majority of cells are neutrophils, but mononuclear cells also are usually increased.

c. The protein concentration is increased and the fluid frequently clots if not submitted in EDTA.

7. Infectious arthropathies are more common in large animal species than in small animals. Organisms usually are not observed cytologically, and confirmation of infection depends on microbiological culture or serology (e.g., Lyme disease, ehrlichiosis).

8. Nonseptic inflammation is usually immune-mediated (e.g., systemic lupus erythematosus, rheumatoid arthritis) but nonimmune-mediated inflammatory arthropathies also occur (e.g., chronic hemorrhagic arthropathies, crystal-induced arthropathies). The nucleated cell count is moderately to markedly elevated, with the range overlapping that of infectious arthropathies. Neutrophils are the predominant cell type.

9. Avian gout may have synovial aspirates with chalky, white, particulate matter. The needle-like, crystalline structure of uric acid is best appreciated in unstained preparations viewed

www.Ebook777.com

microscopically under polarized light. Uric acid crystals may dissolve in aqueous solutions during staining. Heterophils also may be present.

10. Hemarthrosis (e.g., trauma, coagulopathy) is recognized by uniformly bloody fluid during initial specimen collection, by microscopic evidence of erythrophagocytosis when the hemorrhage is more than a few hours old, and by xanthochromia (orange discoloration) visible grossly and hemosiderin and hematoidin visible microscopically when hemorrhage is more chronic.

a. Hemorrhage typically incites inflammation, so the cell count is increased and the number of neutrophils is increased.

b. Samples with iatrogenic hemorrhage transported in the mail (i.e., a delay of more than several hours between sample collection and analysis) may be confused with pathologic hemorrhage because platelets degenerate in old samples and erythrophagocytosis can occur *in vitro*.

11. Eosinophilic arthropathies are rare, but instances have been reported and assumed to be due to a hypersensitivity reaction.

12. Neoplasms involving joints are very rarely diagnosed by synovial fluid analysis.

VI. CEREBROSPINAL FLUID (CSF)

Although rarely diagnostic by itself, CSF analysis, in combination with neurologic examination and specialized diagnostic tests, is a useful adjunct in diagnosis of central nervous system diseases.

A. General considerations

1. CSF analysis must be performed as soon as possible, preferably within 30 to 60 minutes, after collection. Because of the low protein concentration of CSF, cells undergo rapid degeneration and lysis.

2. If the specimen cannot be processed within an hour, cells can be preserved by adding an equal volume of 40% ethanol to a portion of the CSF. Addition of autologous serum to CSF (one drop serum/0.25 mL CSF) also has been shown to preserve cells adequately for up to 48 hours when held at 4°C. Dilution of the CSF must be factored in when determining cell counts. A separate portion of CSF must be reserved unpreserved/undiluted for protein determination, which is more stable.

B. Gross appearance

1. Normal CSF is clear and colorless and it does not clot.

2. Bright red or pink fluid is caused by hemorrhage into CSF. Hemorrhage caused by a traumatic tap is much more common than true hemorrhage. A traumatic tap (iatrogenic hemorrhage) can be recognized as follows:

a. During sampling, a streak of blood is seen in clear CSF with eventual clearing as more fluid is collected.

b. Centrifugation of the CSF results in clear supernatant fluid and a red pellet at the bottom of the tube.

c. In a smear of the fluid, platelets may be visible and erythrophagocytosis is not evident.

3. True pathologic hemorrhage into the subarachnoid space usually is associated with xanthochromia, a yellow-orange discoloration caused by free bilirubin produced as an erythrocyte breakdown product by approximately 48 hours after hemorrhage. Cytologically, hemorrhage is recognized by erythrophagocytosis and/or hemosiderin and hematoidin within macrophages.

4. Turbidity or cloudiness is caused by suspended particles in the fluid.

a. Cells, usually more than 500 cells/μL, cause turbid CSF. Such specimens also may contain fibrinogen and clot. They should be submitted in an EDTA tube to prevent coagulation.

b. Bacteria and fungi can contribute to increased CSF turbidity.

c. Aspirated fat can cause turbidity of CSF.

Free ebooks ==> www.Ebook777.com

C. Total nucleated cell count

1. Cellularity of CSF is generally too low for use of automated cell counters.

2. Cell counts usually are done manually and the CSF is loaded undiluted onto a hemacytometer. Erythrocytes and nucleated cells are each counted in all nine large squares and the numbers are multiplied by 1.1 to determine the number of cells/μL.

3. Normal CSF contains fewer than nine nucleated cells/μL. An increased CSF cell count is termed pleocytosis.

4. Erythrocytes are not normally present in CSF.

a. The presence of erythrocytes indicates blood contamination during collection or pathologic hemorrhage, which must be differentiated to interpret nucleated cell counts and protein content correctly.

b. Low levels of blood contamination (RBC count less than or equal to 13,200/μL) do not significantly alter CSF nucleated cell count or protein concentration.

c. Formulas for correcting cell count and protein concentration for blood contamination are unreliable.

D. Protein

1. The majority of protein in CSF is albumin.

2. The methods used to determine serum protein concentration (e.g., refractometry or chemical methods) are too insensitive to measure the low protein concentration of CSF.

3. Various sensitive methods are available to measure very low protein concentrations. However, these tests vary in their ability to detect albumin and globulins. Therefore, reference intervals differ depending on the particular test used to measure proteins. Thus, reference intervals for one laboratory may not be valid for tests performed at another laboratory.

4. Urine protein dipsticks (reagent strips) measure protein at concentrations typically present in CSF and can be useful as an initial screening or semiquantitative test of CSF protein. Both false-positive and false-negative tests occur at dipstick readings of trace or 1+, but a dipstick reading of 2+ or greater reliably indicates increased CSF protein concentration.

5. Semiquantitative methods for measuring immunoglobulin levels (e.g., Pandy test, Nonne-Apelt test) cause precipitation of globulins, which is graded subjectively. Precipitation does not occur in normal CSF.

6. Increased CSF protein concentration is caused by hemorrhage (iatrogenic or pathologic), increased permeability of the blood-brain or blood-cerebrospinal fluid barrier, increased protein synthesis within the CSF, or tissue degeneration.

a. CSF protein concentration is increased in inflammation, hemorrhage, and tissue degeneration.

b. The increased CSF protein is accompanied by an increased CSF cell count in inflammation and hemorrhage.

c. Increased CSF protein concentration may not be associated with an increase in cellularity (albuminocytologic dissociation) in degenerative or neoplastic diseases and occasionally in viral infections.

7. CSF albumin concentration, usually determined by electrophoresis, can be used to evaluate the integrity of the blood-brain barrier because albumin in CSF is derived from plasma.

a. Because CSF albumin concentration varies with serum albumin concentration, the albumin quotient (AQ) eliminates the variable of the serum albumin in assessing blood-brain barrier function.

b. $AQ = (\text{CSF albumin} \div \text{serum albumin}) \times 100$.

c. AQ greater than 2.35 is increased and suggests an altered blood-brain barrier.

E. Cytologic examination

1. Cytologic examination should be performed on all CSF specimens because CSF samples with normal cell counts can have abnormalities in cell type or morphology.

www.Ebook777.com

2. The cellularity of most CSF specimens is very low, necessitating the use of some type of concentration technique to increase cellularity. Cytocentrifugation is the most common technique used in laboratories. Other methods include slow-speed centrifugation with resuspension of the cellular pellet with a drop or two of autologous serum prior to preparing smears, sedimentation using glass or plastic cylinders (e.g., syringe barrel attached to glass slide), and membrane filtration.
3. Normal CSF is comprised almost exclusively of mononuclear cells. Small lymphocytes predominate; monocytoid cells are less numerous. With the newer concentration methods, a few neutrophils are accepted as normal.
4. Neutrophilic pleocytosis occurs with most bacterial infections, certain viral infections (e.g., feline infectious peritonitis [FIP], eastern equine encephalitis [EEE]), some tumors (particularly meningioma), mycotic infections, steroid-responsive meningitis, and occasionally in granulomatous meningoencephalomyelitis.
5. Lymphocytic/monocytic pleocytosis suggests viral infection, degenerative CNS diseases, listeriosis, or necrotizing meningoencephalitis of Pugs and Maltese dogs. Lymphocytes predominate in most cases of granulomatous meningoencephalomyelitis. Mononuclear cells predominate infrequently in cases of bacterial meningoencephalomyelitis.
6. Eosinophils have been observed in CSF in association with parasitic infection, some fungal infections (e.g., cryptococcosis), and idiopathic steroid-responsive conditions.
7. Neoplastic cells are seen rarely in CSF except in cases of neural lymphoma. Most cases of CNS neoplasia have a mild increase in CSF protein concentration. The nucleated cell count is within the reference interval or increased slightly. In some cases, the CSF is completely normal.
8. With the exception of *Cryptococcus neoformans*, fungi are rarely seen in CSF in mycotic infections.

F. Special tests
1. The activities of creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) have been measured as indicators of CNS disease. None has been found to be sensitive, specific, or of prognostic value. Contamination of CSF with epidural fat or dura can contribute to increased CK activity in CSF.
2. CSF electrolyte concentration and osmolality should be measured concurrently with blood levels. Comparison of CSF to blood values is useful in identifying salt poisoning and other disorders involving electrolyte imbalances.
3. Culture, serology, fluorescent antibody examination, and other techniques to detect and identify infectious agents can be performed on CSF samples to confirm the diagnosis of CNS infection.

VII. RESPIRATORY SYSTEM

Cytology is a useful ancillary test for diagnosis of respiratory tract disorders, particularly when used in conjunction with history, physical examination, radiography, and rhinoscopy or bronchoscopy.

A. Nasal exudates and masses
1. Except for collection of nasal exudates, examination of the nasal cavity and collection of specimens require general anesthesia and endotracheal intubation with an inflated cuff to prevent aspiration of exudates, washing fluid, or blood.
2. Sampling techniques include collection of discharges with cotton swabs, flushing with sterile saline, and aspiration. Swabbing is of limited usefulness unless etiologic agents or tumor cells are within surface material, which is not typically the case.
3. Direct smears may be made from flush fluid if abundant material is obtained. If the fluid is only slightly turbid or contains scant particulate material, smears should be made from centrifuged sediment. Tissue fragments obtained from flushing may be used for imprints, squash preparations, or histopathology if they are large enough.

Free ebooks ==> www.Ebook777.com

4. Normal cytologic findings
   a. Normal cells include squamous epithelial cells (outer nasal cavity and oropharynx) and ciliated columnar epithelial cells.
   b. A mixed bacterial population is present and bacteria frequently are adherent to squamous cells. *Simonsiella* sp., a large stacked, rod-shaped organism specific to the oropharynx, is commonly seen in smears.
5. Inflammation
   a. Purulent inflammation is usually associated with bacterial or fungal infections. The presence of a monomorphic population of bacteria and bacteria phagocytosed by neutrophils is suggestive of bacterial infection. Oronasal fistulas caused by tooth root abscesses can cause primary bacterial rhinitis; however, most bacterial infections are secondary to deeper lesions such as tumors and foreign body reactions, or to viral infections such as canine distemper, FeLV, and FIV.
   b. Fungal infections usually are associated with pyogranulomatous to granulomatous inflammation.
      (1) The fungal infection most often diagnosed by cytology of nasal exudate is cryptococcosis.
      (2) The most common hyphal fungi that cause nasal infections are *Aspergillus* sp. and *Penicillium* sp. They produce characteristic fuzzy white to green growths on the nasal mucosa. In cytologic smears, the hyphae of both organisms are 4 to 6 µm wide with parallel sides, septa, and branching.
   c. Rhinosporidiosis is an infection with *Rhinosporidium seeberi*, an organism of uncertain classification (i.e., fungus vs. protozoa vs. alga).
      (1) This organism causes development of unilateral, red-brown, polypoid nasal masses with characteristic yellow-white stippling.
      (2) The organisms are seen in varying numbers within an exudate comprised primarily of neutrophils. These organisms appear as round to oval spores that are 5 to 10 µm in diameter with a thin, slightly refractile wall and red-pink, spherical, internal, globular structures. Rarely, large sporangia (more than 100 µm in diameter) containing numerous endospores are present in cytologic preparations.
   d. Eosinophilic inflammation usually is attributed to hypersensitivity reactions or parasitic conditions but also may be associated with fungal infections and neoplasia.
6. Nasal masses
   a. Nasal tumors occur more commonly in dogs and cats than in large animals. The majority of these neoplasms are malignant.
   b. The diagnosis of nasal tumors frequently is complicated by secondary inflammation, infection, and necrosis. Thus, surface material may not be representative of the underlying neoplasm.
   c. Carcinomas are more common than sarcomas. These include adenocarcinomas, squamous cell carcinomas, and undifferentiated carcinomas. Cytologically, they consist of clusters or sheets of round or polyhedral cells with a variable nuclear:cytoplasmic ratio, anisocytosis, and anisokaryosis.
   d. The most common connective tissue tumors of the nasal cavity are fibrosarcoma, osteosarcoma, and chondrosarcoma. Except for round cell tumors, mesenchymal tumors do not exfoliate readily. The cells tend to be distributed singly or in small clumps and they vary in appearance. They may be oval, stellate, or spindle-shaped. In addition, neoplastic cells are frequently difficult to differentiate cytologically from reactive fibroblasts. Therefore, histopathology is usually required for a definitive diagnosis.
   e. Round cell tumors of the nasal cavity include lymphoma, mast cell tumor, and transmissible venereal tumor (TVT).
      (1) Lymphoma is characterized by a monomorphic population of medium- to large-sized lymphocytes.

www.Ebook777.com

(2) Mast cell tumors consist of numerous mast cells admixed with variable numbers of eosinophils.

(3) TVT is a tumor of genital tissues and is spread by transplantation of intact tumor cells. Because of the social habits of dogs, this genital tumor may occur in the nose.

f. Ethmoid hematomas (hemorrhagic nasal polyps) of horses are comprised of erythrocytes, leukocytes, macrophages containing phagocytosed erythrocytes and/or hemosiderin, and hematoidin crystals.

g. Nasal polyps consist of fibrovascular tissue lined by epithelium. Because they are comprised of a mixture of tissues arranged in a characteristic architectural pattern, histopathology is required for diagnosis.

B. Tracheo-bronchoalveolar cytology

1. Bronchoalveolar cytology is a useful test in the diagnostic evaluation of an animal with unexplained chronic cough or bronchoalveolar disease.

2. Disorders confined to the interstitium cannot be diagnosed by tracheal/bronchoalveolar washes, which collect material from airways only.

3. Transtracheal/endotracheal wash

a. Warm, sterile saline (1 to 2 mL/10 lb body weight) devoid of bacteriostatic agents is infused via a catheter inserted through an endotracheal tube or between tracheal rings until the animal starts to cough or until the entire fluid volume is injected.

b. Gentle, negative pressure is applied to the syringe immediately to recover a portion of the fluid.

4. Bronchoalveolar lavage is collected through a fiberoptic bronchoscope inserted into a small bronchus.

5. Bronchial brushings are collected by brushing the bronchial mucosa or focal lesions with an endoscopic brush.

6. If the sample will not be processed within 30 to 60 minutes of collection, smears should be made because cells lyse rapidly in protein-poor fluids.

a. Direct smears can be made if the fluid is turbid or contains abundant particulate material from which blood-type and squash preparations can be made, respectively.

b. If the fluid is clear or contains only fine particulate material, smears should be made from centrifuged sediment. Because of the low protein content of the fluid, cell preservation is improved if the sediment material is resuspended in 1 or 2 drops of serum rather than saline.

7. Normal constituents

a. Mucin is a pale blue to pink, finely granular or fibrillar background material. Curschmann's spirals are twisted strands of mucin that represent casts of small bronchioles and occur in any condition that causes chronic over-production of mucin.

b. Epithelial cells

(1) Ciliated epithelial cells are usually columnar but may also be cuboidal. Cilia resemble a pink fringe at one end of the cell. The nucleus is located at the opposite end of the cell.

(2) Nonciliated cuboidal cells are seen less frequently.

(3) Goblet cells are mucus-producing columnar cells with numerous bright pink, mucin-filled, cytoplasmic granules. They are not seen commonly but may be increased in chronic conditions associated with increased mucus production.

c. Alveolar macrophages have a round or oval nucleus and moderate to abundant, blue-gray cytoplasm. Along with epithelial cells, they are the most common cells seen in samples from clinically healthy animals. Alveolar macrophages increase in number in most inflammatory conditions. Their cytoplasm becomes more abundant and vacuolated and may contain phagocytosed cell debris when inflammation is present.

8. Pharyngeal contamination is indicated by the presence of squamous epithelial cells, frequently in association with adherent mixed bacteria.

Free ebooks ==> www.Ebook777.com

a. Nearly incontrovertible evidence of oropharyngeal contamination is the presence of *Simonsiella* sp. organisms, which are specific to the oropharynx.

b. Pharyngeal contamination occurs when the catheter is misdirected upward or when excessive coughing drives the wash fluid up into the pharynx, after which it is reaspirated.

c. In rare instances, squamous cells and mixed bacteria, including *Simonsiella* sp., are seen in smears from cases of aspiration pneumonia.

9. Purulent inflammation

a. Purulent inflammation occurs most commonly with infectious diseases, but noninfectious diseases that cause tissue necrosis (e.g., neoplasms) also can produce purulent inflammation.

b. Increased amounts of mucin and macrophages also are usually present.

c. Infectious agents may be observed; however, their absence does not exclude the possibility of infection. Specimens with many neutrophils should be cultured to eliminate the possibility of infection more definitively.

d. Neutrophil degeneration is difficult to evaluate in these washes because the low protein content of wash fluid results in cellular degeneration, even in nonseptic conditions.

e. Bacterial infections in animals are most commonly caused by Gram-negative rods. In true bacterial infections, as compared to pharyngeal contamination, the bacteria are usually a single population and are phagocytosed by neutrophils as well as free in the background of the smear.

10. A variety of fungi cause pulmonary lesions (see section on masses, lesions, and tissues for more detailed morphologic descriptions of these organisms).

a. The inflammatory infiltrate typically consists of neutrophils and variable numbers of macrophages (pyogranulomatous inflammation). Multinucleated giant cells also may be present.

b. The lung is the primary site of infection with *Blastomyces dermatitidis*. Blastomycosis is most common in the dog.

c. *Cryptococcus neoformans* and *Histoplasma capsulatum* are less frequent causes of pneumonia.

d. Various hyphal fungi cause lower respiratory tract infection, with the most common being *Aspergillus* sp.

11. Eosinophilic inflammation usually is associated with hypersensitivity reactions and parasitic conditions but eosinophils also may be a significant component of inflammatory reactions caused by various infectious agents and neoplasms.

a. Eosinophil granules frequently appear muddy rust-brown in tracheal/bronchoalveolar wash specimens and may be difficult to identify except in thin portions of smears.

b. Several studies of clinically normal cats found eosinophils to be a significant component of the cell population in tracheopulmonary washes (approximately 20% to 25% eosinophils). Consequently, the presence of eosinophils in specimens from cats must be interpreted with respect to clinical signs and radiographic findings.

12. Chronic hemorrhage occurs in many conditions, including trauma, infectious diseases, cardiac disease, exercise-induced pulmonary hemorrhage, and neoplasia. It is recognized by the presence of alveolar macrophages containing phagocytosed erythrocytes, hemosiderin, and hematoidin.

13. Neoplastic cells are observed infrequently in tracheopulmonary washes.

a. In most cases, metastatic tumors are confined to the interstitium and are not collectable by wash procedures until they have invaded airways.

b. Neoplasms that have invaded airways and primary carcinomas of the bronchopulmonary tree may exfoliate tumor cells into the wash solution.

(1) Carcinomas are typically comprised of large, basophilic, polyhedral cells that usually have a high nuclear:cytoplasmic ratio. The cells are distributed singly and in clusters.

(2) Sarcomas, except for lymphoma, typically yield very few cells in respiratory tract washes. Biopsy or fine-needle aspiration usually is required for diagnosis.

www.Ebook777.com

VIII. VAGINAL CYTOLOGY

The vaginal smear is a useful method to determine the optimal time for breeding a bitch and may aid in diagnosis of some inflammatory and neoplastic disorders of the reproductive tract.

A. Collection of cells
- **1.** Cells are obtained by swabbing the caudal vagina with a moist cotton swab. The swab is directed craniodorsally when entering the vaginal vault to avoid the clitoral fossa, which usually contains keratinized cells that would alter cytologic findings.
- **2.** The smear is prepared by gently rolling the swab on a slide.

B. Normal cells
- **1.** Basal cells comprise the deepest layer of the epithelium and, thus, are rarely seen in vaginal smears.
- **2.** Parabasal cells are the smallest epithelial cells normally seen in vaginal smears. They are small, uniformly sized cells with a round nucleus and small amount of cytoplasm. They may exfoliate in sheets in prepubertal dogs.
- **3.** Intermediate cells are approximately twice the size of parabasal cells. Their nuclei are similar to those of parabasal cells. As intermediate cells enlarge, their cell margins become angular and folded.
- **4.** Superficial cells are the largest cells. They have faded or pyknotic nuclei or are anucleate. These cornified cells have angular or folded margins.

C. Cytologic characteristics of the estrous cycle
- **1.** Proestrus
  - **a.** This is the beginning of the estrous cycle. As the concentration of estradiol increases, epithelial cells proliferate and erythrocyte diapedesis occurs.
  - **b.** In early proestrus, all types of epithelial cells as well as erythrocytes and neutrophils are present.
  - **c.** At late proestrus, neutrophils decrease in number and intermediate and superficial cells become predominant.
  - **d.** Bacteria may be present throughout the cycle and are adherent to epithelial cells.
- **2.** Estrus
  - **a.** Superficial/keratinized cells comprise 90% or more of the cell population. Many of these cells are anucleate.
  - **b.** Neutrophils are absent. Erythrocytes may be present in variable numbers.
  - **c.** Bacteria, both free and adherent to epithelial cells, may be present.
  - **d.** The background of the smear usually is clear of debris.
- **3.** Diestrus
  - **a.** The number of superficial cells abruptly decreases. Intermediate and parabasal cells increase, often constituting more than 50% of the cell population.
  - **b.** Neutrophils increase in number and erythrocytes may be present again.
  - **c.** Usually it is not possible to distinguish proestrus from diestrus on a single vaginal smear.
- **4.** Anestrus
  - **a.** Parabasal and intermediate cells predominate.
  - **b.** Neutrophils and bacteria may be present in small numbers.

D. Reproductive diseases
- **1.** Vaginitis and metritis
  - **a.** Vaginitis may be primary (e.g., *Brucella canis*, herpesvirus, *Mycoplasma* sp.) but is more commonly secondary to a primary vaginal anomaly.

Free ebooks ==> www.Ebook777.com

b. Metritis usually follows parturition. The bitch is usually febrile and has a malodorous uterine discharge.
c. Smears contain large numbers of neutrophils (often degenerate) that may contain phagocytosed bacteria within their cytoplasm.
d. Inflammation may be difficult to differentiate from proestrus or diestrus, but neutrophils decrease during normal cycling, whereas they remain constant with inflammation.
e. Macrophages and lymphocytes may be numerous in chronic inflammation.

2. Subinvolution of placental sites
a. This condition is caused by retention of placental tags at implantation sites so that the blood supply is maintained to the tissue tags.
b. It occurs most commonly in young bitches after the first whelping and consists of a bright red, bloody discharge that persists for weeks to months after parturition.
c. The cytologic smear consists of blood cells.

3. Neoplasia
a. Some vaginal neoplasms may be diagnosed by vaginal smears.
b. Transmissible venereal tumor, squamous cell carcinoma, lymphoma, and transitional cell carcinoma that has invaded the vagina are the most common tumors that can be diagnosed cytologically.

IX. EQUINE ENDOMETRIAL CYTOLOGY

Endometrial cytology is used to determine whether inflammation or infection is present to evaluate breeding soundness.

A. Method of collection
1. A sterile collection device (swab or flushing catheter) is guarded by a gloved hand, guided through the vulva and vagina, and advanced through the cervix into the uterus.
2. Fluid lavage is done by introducing a sterile insemination pipette into the uterus, flushing 50 mL sterile saline into the uterus with a 60-mL syringe, and immediately applying negative pressure to the syringe to collect a sample of fluid (usually 1 to 5 mL).
3. Preparation of smears depends on the method of collection.
a. Swabs are rolled onto a slide to make direct smears.
b. Flush samples must be centrifuged and smears are prepared from the sediment material.

B. Cytologic examination
1. Endometrial epithelial cells are ciliated or nonciliated, columnar cells distributed singly, in clusters, or in large sheets. They have small dark oval nuclei located at the basal aspect of the cell opposite the cilia.
2. Erythrocytes are present postpartum, following endometrial biopsy, in acute inflammatory conditions, and following intrauterine trauma.
3. The presence of squamous cells in endometrial smears indicates vaginal contamination.
4. Inflammatory cells are not normally present in endometrial smears.
a. Neutrophils in numbers greater than 2% of the nucleated cell population or in a ratio greater than one neutrophil/40 endometrial cells indicates active inflammation.
b. Lymphocytes and macrophages may be seen in chronic inflammation.
c. The presence of eosinophils has been associated with pneumovagina and pneumouterus.
d. In barren mares, endometrial specimens occasionally may exhibit inflammation, but infectious agents are infrequent to rare.

5. Infectious agents
a. Infectious agents rarely are observed in most equine endometrial cytology specimens.
b. Bacteria, yeasts (*Candida* sp.), and fungus (*Aspergillus* sp.) have been observed in a small percentage of endometrial specimens.

www.Ebook777.com

REFERENCES

Bienzle D, McDonnell JJ, Stanton JB: 2000. Analysis of cerebrospinal fluid from dogs and cats after 24 and 48 hours of storage. *J Am Vet Med Assoc* 216:1761–1764.

Bouvy BM, Bjorling DE: 1991. Pericardial effusion in dogs and cats. Part I. Normal pericardium and causes and pathophysiology of pericardial effusion. *Compend Contin Educ Pract Vet* 13:417–421, 424.

Campbell TW: 1995. Avian Hematology and Cytology, 2nd ed. Ames, IA, Iowa State University Press.

Cowell RL, Tyler RD (eds): 2002. Diagnostic Cytology and Hematology of the Horse, 2nd ed. St. Louis, Mosby, Inc.

Cowell RL, Tyler RD, Meinkoth JH (eds): 1999. Diagnostic Cytology and Hematology of the Dog and Cat, 2nd ed. St. Louis, Mosby, Inc.

Fossum TW: 1993. Feline chylothorax. *Compend Contin Educ Pract Vet* 15:549–567.

Fossum TW, Wellman M, Relford RL, et al: 1993. Eosinophilic pleural or peritoneal effusions in dogs and cats: 14 cases (1986–1992). *J Am Vet Med Assoc* 202:1873–1876.

Furr MO, Tyler RD: 1990. Cerebrospinal fluid creatine kinase activity in horses with central nervous system disease: 69 cases (1984–1989). *J Am Vet Med Assoc* 197:245–248.

Green EM, Constantinescu GM, Kroll RA: 1993. Equine cerebrospinal fluid: Analysis. *Compend Contin Educ Pract Vet* 15:288–302.

Hirschberger J, DeNicola DB, Hermanns W, et al: 1999. Sensitivity and specificity of cytologic evaluation in the diagnosis of neoplasia in body fluids from dogs and cats. *Vet Clin Pathol* 28:142–146.

Hurtt AE, Smith MO: 1997. Effects of iatrogenic blood contamination on results of cerebrospinal fluid analysis in clinically normal dogs and dogs with neurologic disease. *J Am Vet Med Assoc* 211:866–867.

Jackson C, de Lahunta A, Divers T, et al: 1996. The diagnostic utility of cerebrospinal fluid creatine kinase activity in the horse. *J Vet Intern Med* 10:246–251.

Jacobs RM, Cochrane SM, Lumsden JH, et al: 1990. Relationship of cerebrospinal fluid protein concentration determined by dye-binding and urinary dipstick methodologies. *Can Vet J* 31:587–588.

Malark JA, Peyton LC, Galvin MJ: 1992. Effects of blood contamination on equine peritoneal fluid analysis. *J Am Vet Med Assoc* 201:1545–1548.

Muñana KR, Luttgen PJ: 1998. Prognostic factors for dogs with granulomatous meningoencephalomyelitis: 42 cases (1982–1996). *J Am Vet Med Assoc* 212:1902–1906.

Padrid PA, Feldman BF, Funk K, et al: 1991. Cytologic, microbiologic, and biochemical analysis of bronchoalveolar lavage fluid obtained from 24 healthy cats. *Am J Vet Res* 52:1300–1307.

Radaelli ST, Platt SR: 2002. Bacterial meningoencephalomyelitis in dogs: A retrospective study of 23 cases (1990–1999). *J Vet Intern Med* 16:159–163.

Savary KCM, Sellon RK, Law JM: 2001. Chylous abdominal effusion in a cat with feline infectious peritonitis. *J Am Anim Hosp Assoc* 37:35–40.

Stalis IH, Chadwick B, Dayrell-Hart B, et al: 1995. Necrotizing meningoencephalitis of Maltese dogs. *Vet Pathol* 32:230–235.

Sweeney CR, Russell GE: 2000. Differences in total protein concentration, nucleated cell count, and red blood cell count among sequential samples of cerebrospinal fluid from horses. *J Am Vet Med Assoc* 217:54–57.

Free ebooks ==> www.Ebook777.com

Welles EG, Pugh DG, Wenzel JG, et al: 1994. Composition of cerebrospinal fluid in healthy adult llamas. *Am J Vet Res* 55:1075–1079.

Welles EG, Tyler JW, Sorjonen DC, et al: 1992. Composition and analysis of cerebrospinal fluid in clinically normal adult cattle. *Am J Vet Res* 53:2050–2057.

Whitney MS, Roussel AJ, Cole DJ: 1999. Cytology in bovine practice: Solid tissue, pleural fluid, and peritoneal fluid specimens. *Vet Med* 94:277–278, 280–281, 283–286, 288–289.

www.Ebook777.com

Free ebooks ==> www.Ebook777.com

CHAPTER 13

GENERATING AND INTERPRETING TEST RESULTS: TEST VALIDITY, QUALITY CONTROL, REFERENCE VALUES, AND BASIC EPIDEMIOLOGY

Paula M. Krimer, DVM, DVSc, DACVP

I. MEDICAL DECISIONS ARE MADE BASED ON THE COMPARISON OF PATIENT LABORATORY VALUES WITH THOSE OF A REFERENCE GROUP. FOR ALL VALUES TO BE CLINICALLY USEFUL, FOUR DISTINCT CRITERIA MUST BE MET.

A. Test validity. The test itself must be valid and measure the analyte of interest over a range of values with minimal interference from other substances. Sources of error should be minimized.

B. Quality control. The test must be run correctly. The laboratory must maintain stringent quality control to constantly ensure the test is run correctly and report the best possible measure of the analyte.

C. Reference intervals. The test result is compared to known values from healthy patients. The reference intervals must represent a sufficiently large and appropriate demographic for comparison, and must be created using the same test methodology.

D. Basic epidemiology. The selected analyte must have a strong association with a medical condition such that the test will have few false-positive and false-negative results. Test selection is critical and should vary with the pre-test probability of disease.

II. TEST VALIDITY

A. The test should measure the analyte of interest over a range of values with minimal interference from other substances.

B. Because reagents and instruments used to perform laboratory tests on animal specimens often are marketed solely for testing human specimens, one *cannot* assume that a procedure will produce valid results on samples from other species.

1. Laboratories in human settings do not have equipment calibrated for animal samples and do not validate tests for animals, and reference intervals cannot be applied to animals. *Do not use human laboratories for analysis of veterinary specimens.*

2. Test validation, especially those involving antibodies, can be challenging in non-domestic species because reagents may not cross react.

www.Ebook777.com

C. Protocols to validate an assay are described in most clinical chemistry textbooks. Typical components of the validation procedure include assessment of the following:

1. Analytical specificity. This is the ability of the assay to measure the analyte in question in the presence of potentially interfering substances. Analytical specificity can be evaluated by adding known or suspected interfering substances to the sample or by using samples from patients with a known condition (e.g., lipemia, hemolysis, bilirubinemia).

2. Lower limit of detection or analytical sensitivity. This is the smallest amount of an analyte that the test can detect. The lower limit of detection often is assessed by running a series of dilutions of a known standard.

3. Upper limit of detection. This is the greatest amount of the analyte that the test can reliably measure or after which the results are no longer reliable.

4. Linearity. A test which yields results directly proportional to the concentration of the analyte has good linearity. Most tests are linear over a specific reportable range of values, which are bound by the reportable upper and lower limits of detection. Those which are not linear can sometimes be modified by mathematical transformation and become suitable for laboratory use.

5. Accuracy. The accuracy of a laboratory result is how close the test result is to the 'true value' of the analyte. This is usually the degree of agreement between the test result and a gold standard or known value.

a. For most common analytes, accuracy is determined using a designated analytical reference method and calibration materials (reagents). For other analytes, such values may not exist because of the lack of agreement among laboratories on a suitable analytical reference method. This is a problem in veterinary laboratory medicine in general, and even more problematic for non-domestic species.

b. During the validation process, analytical accuracy is determined by running known amounts of the analyte and comparing the results to expected values, performing recovery studies, or using split samples and comparing a new test method to an existing test method of known accuracy.

6. Precision. The precision of a laboratory method is its reproducibility. A test can have a high degree of precision (always gives the same value), but a low degree of accuracy (the value is incorrect).

a. Laboratory tests vary greatly in their precision. Pre-analytical factors (see section D, 1 below) as well as analytic factors such as interfering substances, assay method, instrumentation, and skill of the technician influence precision.

b. A common means to measure and report the precision of a quantitative laboratory method is the coefficient of variation (CV). The CV is expressed as a percentage and can be determined by analyzing the same sample repeatedly. A minimum of 30 repeat samples is required to calculate a CV. The mean and standard deviation of the set of results is calculated, and the CV is expressed by the following formula:

$$\text{CV} = 100 \times (\text{standard deviation} \div \text{mean}).$$

c. With appropriate testing, CVs can be calculated separately for variation within a single set of samples (within run variation) and within sets of samples analyzed from day to day (between-run variation).

d. An ideal test has a CV of less than 5%, with a CV of less than 10% being acceptable for many tests. For some tests, the best CV that can be practically achieved is closer to 20%.

D. Sources of test error can be pre-analytical, analytical, or post-analytical.

1. Pre-analytical errors are caused prior to analysis. These are the *most* common errors made by practitioners and also the easiest to prevent or rectify.

2. Common pre-analytic errors include the following:

a. Incorrect site of sample procurement (e.g., arterial vs. venous for blood gases)

Free ebooks ==> www.Ebook777.com

b. Incorrect or poor sampling technique (e.g., traumatic venipuncture for coagulation assays, blood clotting inside syringe)
c. Incorrect sample container or anticoagulant (e.g., EDTA tube for a biochemistry panel)
d. Incorrect anticoagulant-to-blood ratio or insufficient sample volume (e.g., under-filling tube for coagulation assays or filling tube with too little blood from small species of animals)
e. Inadequate processing prior to submission (e.g., sending serum on the clot to the laboratory without centrifuging, not making blood and fluid smears)
f. Poor packaging (e.g., sample breaking in transit, exposing blood smears to formalin fumes)
g. Incorrect specimen identification (e.g., not labeling or mislabeling the sample)
h. Incorrect test identification (e.g., marking an incorrect test on the submission form)
i. Sample degradation (e.g., storing the sample too long prior to analysis)
j. Temperature fluctuations (e.g., freezing in winter or excessive heating in summer)

3. Analytical errors arise during analysis of the sample in the commercial laboratory or when using in-clinic machines. These errors can be random (occurring only once) or systematic (due to a general problem). Laboratories must validate a test and maintain strict quality control to minimize analytical errors. Practitioners are responsible for properly calibrating, maintaining, and performing quality control when using in-house analyzers.
4. Post-analytical errors are generally associated with incorrect data entry and generation of reports.

III. QUALITY CONTROL AND QUALITY ASSURANCE

A. The test must be run correctly. The external laboratory or in-clinic laboratory must maintain stringent quality control to constantly ensure the test is run correctly and to report the best possible measure of the analyte.

B. Commercial and institutional laboratories
1. Quality control. Large laboratories should have systematic programs to ensure that test results are valid. These programs often are complex and have numerous components intended to detect potential sources of error in laboratory values.
2. A critical concept of a quality control and quality assurance program is that it is a central part of the daily operation of a laboratory and that detailed records of the quality control system are maintained. Larger laboratories often employ individuals whose sole purpose is to monitor all quality control operations.
3. A laboratory that does not maintain a quality control program cannot ensure its test results are valid and should not be used.
4. Discussion of the details of a quality control program is largely outside the scope of this text; however, a typical program includes the following:
a. Systematic and periodic monitoring and calibration of analytic equipment such as spectrophotometers, electronic cell counters, flow cytometers, centrifuges, refrigerators, and pipettes. These are calibrated and monitored daily or periodically, sometimes by third parties or the original equipment manufacturer.
b. A system to monitor reagent inventory, storage, and handling. This system ensures that reagents are within their expiration dates; reagents do not degrade, expire, or lose sterility; and water quality is maintained.
c. Controls with known ranges of acceptable results are used for each test performed in the laboratory to monitor ongoing test accuracy and precision. Controls are assayed along with patient samples to ensure that the instruments, technicians, and reagents are functioning properly.
d. Graphic plots such as Levey-Jennings charts (Figure 13.1) allow visualization of variation in daily controls for violation of rule-based algorithms (e.g., Westgard Rules or Six Sigma) and are

www.Ebook777.com

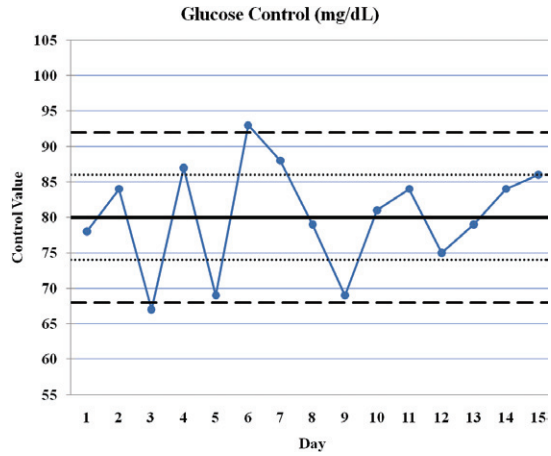


FIGURE 13.1. Example of a Levey-Jennings chart showing a glucose control over a 15-day period. The expected value of 80 mg/dL is indicated by the thick central solid line. The dotted lines are one standard deviation away from the expected mean, and the dashed lines are two standard deviations different from the expected mean.

entered into statistical programs to evaluate the accuracy and precision of control data over time. If the control is statistically outside of a specified interval or a series of controls violates the statistical rules or shows a distinct trend (multi-rule violations based on bias and coefficient of variance), patient values in that test run(s) may be invalid or a general equipment or reagent problem may exist.

e. Maintenance of detailed written records to monitor each of the above components and to document that quality control tasks are being done.

5. Quality Assurance

a. Laboratories often participate in third-party accreditation and/or proficiency programs that verify their adherence to and maintenance of a quality control program and also evaluate the quality of their test results.

b. Accreditation involves a combination of laboratory audits, inspections, and site visits to endorse the quality control system. Selected examples of agencies that administer accreditation programs include the American Association of Veterinary Laboratory Diagnosticians (AAVLD), COLA (previously the Commission on Office Laboratory Accreditation), and the American Association for Laboratory Accreditation (AALA).

c. Proficiency programs are distinct from accreditation programs; they measure the quality of laboratory results. The laboratory periodically receives blinded samples of unknown composition to be assayed and test results are submitted to the agency administering the proficiency program. The laboratory subsequently receives a report comparing its test results to those of other laboratories. Agencies that administer proficiency programs include the Veterinary Laboratory Association (VLA), National Veterinary Service, and National Animal Health Laboratory Network (NAHLN).

d. Selection of a good veterinary laboratory should be based on its internal quality control methods, accreditation by outside agencies, and participation in laboratory proficiency programs. Asking your laboratory about its quality control and quality assurance practices is recommended.

C. In-clinic testing

1. The main advantage of in-clinic testing is to obtain rapid test results, rather than to decrease costs or increase test accuracy. However, inaccurate but rapid test results are not useful and *potentially harmful to the patient*.

Free ebooks ==> www.Ebook777.com

2. Laboratory analyses performed in private veterinary practices are not performed in an optimal setting as outlined above and the likelihood of serious error is much higher. Therefore, it is even more critical that a rigorous quality control program be implemented and maintained.
3. The absence of a quality control program or dedicated quality control personnel for in-clinic analyzers or point-of-care instruments will lead to erroneous results and poor medical decisions for which the veterinarian is legally liable.
4. Newer in-clinic analyzers for some routine hematological and biochemical tests can be quite accurate and reliable in domestic species if properly maintained and supported. Many analyzers have built-in quality control systems that automatically run controls and monitor sensors and fluid-levels.
5. Some analyzers and point-of-care instruments provide results, even when instrumentation indicates that controls have failed. It is improper and potentially harmful to the patient to accept a test result or make medical decisions based on dubious test results that fail internal controls or are assayed with expired reagents.
6. A good in-clinic analyzer is fully supported by the manufacturer with excellent customer service. This process includes the following:
- **a.** Assistance to establish and maintain a quality control program for a given analyzer
- **b.** Appropriate control samples and calibration standards
- **c.** Assistance in validating or transferring reference intervals
- **d.** Trouble-shooting assistance or an assistance hotline
- **e.** A long-term service contract with routine equipment maintenance, repair, or replacement as needed

7. Quality control is a significant but necessary monetary and labor expenditure if in-clinic analyzers are to be used. In a practice setting where the costs of seldom-used reagents and quality control procedures cannot be amortized over a large volume of samples, a modest quality control program can double the average cost-per-patient test result.
8. When veterinarians consider purchasing equipment for in-house laboratory analyses, it is important to include the costs of a quality control program, the manufacturer's support and service contract, and required personnel to perform the equipment maintenance, laboratory testing, and quality control.
9. Reference intervals should ideally be established for each individual in-clinic analyzer; those provided by the manufacturer may not be applicable without transfer validation studies. In addition, test results and reference intervals between species and for non-domestic species should never be considered accurate without proper test validation and creation of species-specific reference intervals. See section IV, F for a more thorough discussion on transfer of reference intervals.

IV. REFERENCE INTERVALS

A. To make medical decisions, patient test results are compared to known values from healthy patients. The reference intervals must represent a sufficiently large and appropriate demographic for comparison, and must be created using the same test methodology.
1. The term reference value is preferred over the term 'normal value' because of the difficulty in defining normality.
2. The term reference range is incorrect because it refers to the difference between the upper and lower limits of a reference interval and is a single whole number. For example, if the reference interval for glucose in adult dogs is 76 to 119 mg/dL, the range of the reference interval is 43 mg/dL (i.e., 119 − 76 = 43).
3. Reference intervals are specific to the *test method* and *reference group*. They cannot be transferred between laboratories without validation, even when the equipment and test method are the same.

www.Ebook777.com

The use of published reference intervals for diagnostic decisions is not advised, especially if measured with a different instrument or different reagents, or the reference group is not the same as that of the patient.

B. The best reference interval for a patient is calculated from a similar group of healthy animals, known as the reference group. Clearly defined demographic, environmental, and physiological parameters are used to select appropriate reference individuals in the group.

1. Demographic parameters for the reference group include:
   - **a.** Species (e.g., hematocrit and MCV are higher in dogs than cats). This is difficult for rare or uncommon exotic or wildlife species.
   - **b.** Breed (e.g., active breeds have higher hematocrit)
   - **c.** Age (e.g., younger animals have lower hematocrit percentage and protein concentration and higher lymphocyte counts)
   - **d.** Sex (e.g., male dogs have higher hematocrit values than females)
2. Environmental and physiological conditions for the reference group include the following:
   - **a.** Diet (e.g., animals on a high-protein diet have a higher BUN concentration)
   - **b.** Fasted or unfasted (e.g., blood glucose and bile acid concentrations are lower following fasting)
   - **c.** Reproductive or lactation status (e.g., decreased hematocrit in pregnant dogs, decreased triglycerides in nursing mares, decreased calcium in lactating cattle)
   - **d.** Level of excitement (e.g., increased hematocrit percentage and lymphocyte count in excited cats)
   - **e.** Body condition (e.g., creatinine concentration is higher in well-muscled animals)
   - **f.** Altitude (e.g., RBC count is increased at high altitudes)
   - **g.** Medications (e.g., corticosteroids increase the neutrophil count and ALP activity)
   - **h.** Season of the year (e.g., some analytes change with the length of day, mating season, external temperature, etc., especially in non-domestic species)

C. Reference intervals apply only to samples collected, handled, and analyzed using a similar test methodology. This includes all aspects of the sample and the analytic method of the test.

1. Specimen collection parameters include the following:
   - **a.** Collection site (e.g., WBC count is higher in capillary than venous blood)
   - **b.** Anticoagulant used (e.g., platelets usually vary with anticoagulants; many biochemical parameters are different in citrated plasma than serum and vary by species)
   - **c.** Sampling time (e.g., time of day, postprandial sampling time for bile acids, post-ACTH administration time)
2. Specimen handling parameters include:
   - **a.** Post-collection interval before testing (e.g., sorbitol dehydrogenase is only stable a few hours)
   - **b.** Storage conditions, including heating, freezing, and thawing (e.g., freezing whole blood causes hemolysis and heating ruins cell morphology)
3. Analytical method parameters include:
   - **a.** The type of machine or instrument, specific reagents, specific test method, biochemical reactions, temperature, and calibration methods must be the same for reference intervals to be valid (e.g., hematocrit and platelet counts of the same blood specimen varied on seven different analyzers; some ALT assays add pyridoxal 5'-phosphate to convert the inactive apoenzyme to a functional holoenzyme, whereas some do not).
   - **b.** Values should be compared only with the same units or should be converted with appropriate conversion factors (e.g., metric international standard units vs. empirical units).

D. Methods to calculate the reference intervals vary with the number of individuals and normality of the patient data, but usually represent the central 95% of the reference group. A variety of statistical methods can be used.

Free ebooks ==> www.Ebook777.com

1. Ideally, at least 120 individuals should be used to calculate the reference interval. In veterinary medicine, this number often is not reached.
2. If more than 40 individuals are in the selected reference group and the values are normally (Gaussian) distributed, the reference interval is calculated using a mean ± 2 standard deviations. This should include 95% of the values.
3. If there are more than 40 individuals and the values are not normally distributed, the non-parametric central 95% is calculated using the rank-percentile method. In this simple calculation, the bottom 2.5% and the top 2.5% of the values are excluded. The advantage of this method is that it is impossible for values not actually measured or non-physiological values to be included in the reference interval.
4. When using either of these techniques, values from 95% of the animals are included; however, values from 5% of the clinically healthy animals are expected to fall outside of the reference interval.
    a. Statistically, this means that even for a healthy animal, one test out of 20 can be expected to be outside the reference intervals.
5. If there are less than 40 reference individuals, the highest and lowest values observed should be used as the high and low reference interval limits.
6. Reference intervals used for the case studies in this text are listed in Tables 13.1, 13.2, and 13.3.

E. The more diverse the reference group parameters, the wider the reference intervals, whereas the more homogenous the population, the narrower the reference intervals.

1. Wide reference intervals for any test parameter are expected when sampling a population of dogs of various age, sex, breed, and health status with an unknown period of fasting, unknown medication status, and an unknown delay in sample analysis.
2. Much narrower reference intervals for any test parameter are expected when sampling a population of non-medicated, fasted, adult, male, healthy beagles with immediate laboratory analysis of the specimens.
3. The narrower reference intervals that are derived from a relatively homogeneous population have greater diagnostic sensitivity in detecting disease (i.e., they facilitate detection of minor deviations from health). However, there are two main disadvantages:
    a. If the reference interval is applied to animals that do not have the same population parameters, incorrect conclusions may be drawn regarding the meaning of the test results. For example, puppies are born with a lower hematocrit and have higher ALP associated with bone growth relative to adults. A puppy may be misdiagnosed with anemia or liver disease if its hematocrit or ALP is evaluated using reference intervals for adult dogs.
    b. Though ideal, it is logistically impossible to determine separate reference intervals for every subpopulation of animals that a typical veterinary laboratory encounters. Awareness of the characteristics of the reference group is paramount in comparing the reference intervals with subpopulations with different demographic, environmental, or physiological parameters.
4. Reference values previously determined on an individual animal produce the narrowest reference intervals.
    a. The patient's previous laboratory values, obtained in a state of health, serve as reference points for the interpretation of future laboratory test results.
    b. This method often is used in studies in which pretest reference intervals are determined on each individual animal before the start of the study.

F. Reference intervals should not be transferred between laboratories unless the analytical method (including instruments and reagents) is the same.

1. Transfer of reference intervals requires specific validation steps. This is usually a comparison of split sample results between an established instrument and a new instrument. Reference intervals may be transferable or require mathematical manipulation after linear regression to correlate the two machines.

www.Ebook777.com

TABLE 13.1
HEMATOLOGY REFERENCE INTERVALS.*

Test	Dog	Cat	Horse	Cow	Units	Conversion factor	SI units
Hematocrit (Hct)	35–57	30–45	27–43	24–46	%	0.01	L/L
Hemoglobin (Hb)	11.9–18.9	9.8–15.4	10.1–16.1	8.0–15.0	g/dL	10	g/L
Red blood cell count (RBC)	4.95–7.87	5.0–10.0	6.0–10.4	5.0–10.0	$\times 10^6/\mu L$	1	$\times 10^{12}/L$
Mean corpuscular volume (MCV)	66–77	39–55	37–49	40–60	fL	Same	
Mean corpuscular Hb (MCH)	21.0–26.2	13–17	13.7–18.2	11–17	pg	Same	
Mean corpuscular Hb concentration (MCHC)	32.0–36.3	30–36	35.3–39.3	30–36	%(g/dL)	10	g/L
Reticulocytes	0.0–1.0	0.0–0.6	0.0–0.0	0.0–0.0	%		
Absolute reticulocyte count	<80	<60	0	–	$\times 10^3/\mu L$	1	$\times 10^9/L$
Platelet count	211–621	300–800	117–256	100–800	$\times 10^3/\mu L$	1	$\times 10^9/L$
Mean platelet volume (MPV)	6.1–10.1	12–18	4.0–6.0	3.5–6.5	fL	same	fL
White blood cell count (WBC)	5.0–14.1	5.5–19.5	5.6–12.1	4.0–12.0	$\times 10^3/\mu L$	1	$\times 10^9/L$
Segmented neutrophils (Seg)	2.9–12.0	2.5–12.5	2.9–8.5	0.6–4.0	$\times 10^3/\mu L$	1	$\times 10^9/L$
Band neutrophils (Band)	0.0–0.45	0.0–0.3	0.0–0.1	0.0–0.1	$\times 10^3/\mu L$	1	$\times 10^9/L$
Lymphocytes	0.4–2.9	1.5–7.0	1.16–5.1	2.5–7.5	$\times 10^3/\mu L$	1	$\times 10^9/L$
Monocytes	0.1–1.4	0.0–0.9	0.0–0.7	0.0–0.9	$\times 10^3/\mu L$	1	$\times 10^9/L$
Eosinophils	0.0–1.3	0.0–0.8	0.0–0.78	0.0–2.4	$\times 10^3/\mu L$	1	$\times 10^9/L$
Basophils	0.0–0.14	0.0–0.2	0.0–0.3	0.0–0.2	$\times 10^3/\mu L$	1	$\times 10^9/L$
Myeloid/erythroid ratio (M/E)	0.75–2.5	0.6–3.9	0.5–1.5	0.3–1.8			

* These reference intervals have been derived from healthy adult animals at the University of Georgia College of Veterinary Medicine and are to be used with the study cases in this textbook (unless otherwise indicated). These reference intervals are not appropriate for interpretation of data from other laboratories.

Free ebooks ==> www.Ebook777.com

TABLE 13.2
HEMOSTASIS REFERENCE INTERVALS.*

Test	Dog	Cat	Horse	Cow	Units
Bleeding time (BT)	1–5	1–5	1–5	1–5	Minutes
Activated clotting time (ACT)	60–100	665	120–190	90–120	Seconds
One-step prothrombin time (OSPT)	5.8–7.9	7.1–10.9	8.2–11.0	–	Seconds
Activated partial thromboplastin time (APTT)	13.1–17.4	11.5–19.9	30–50	–	Seconds
Thrombin clotting time (TCT)	4.2–7.0	4.0–8.7	11.0–20.0	–	Seconds
Fibrin degradation products (FDP)	0–32	0–8	0–16	–	µg/mL
Fibrinogen	150–300	150–300	100–400	100–600	mg/dL
Platelet count	211–621	–	117–256	100–800	$\times 10^3/\mu L$
Factor VIII activity	50–200	–	–	–	% of normal
von Willebrand's factor antigen (vWF:Ag)	60–172	–	–	–	% of normal

*These reference intervals are from the University of Georgia College of Veterinary Medicine and are to be used with the study cases in this textbook (unless otherwise indicated). These reference intervals are not appropriate for interpretation of data from other laboratories.

2. Transfer of reference intervals occurs more frequently with some laboratory tests (e.g., WBC differential count, BUN concentration, total protein concentration) because the methods of analysis are similar or standardized.
3. Transfer of reference intervals with other tests may be difficult or impossible because of differences in equipment, substrate, ionic strength of buffer solutions, and reaction temperatures (e.g., most enzymatic tests).

G. A frequent conundrum in veterinary medicine is that clinicians sometimes are forced to interpret laboratory data in the absence of appropriate reference intervals, including such appropriate basic reference group parameters as species. The use of reference intervals from related species is sometimes used in these instances, but this should be avoided. At minimum, blood from one or several healthy animals of the same species should be submitted for comparison if possible.

V. BASIC EPIDEMIOLOGY

A. To be clinically useful, an abnormal test value must have a high degree of association with the disease condition.

B. Many tests such as biochemistry and serology are continuous values rather than dichotomous outcomes.
1. Usually, the higher the test value, the stronger the association with the disease.
2. A medical decision limit is the abnormal test value at which a clinician makes a diagnosis or therapeutic choice, or at which the prognosis changes.
3. The upper limit of the reference interval may be used as a medical decision limit, but often the chosen value is higher than the upper reference limit (e.g., ALP for cholestasis), or occasionally lower than the lower reference limit (e.g., RBC for anemia).

C. An important concept is that the medical decision limit is *selected*.
1. The value chosen as the medical decision limit is referred to as the cut-off.

www.Ebook777.com

TABLE 13.3
CHEMISTRY REFERENCE INTERVALS.*

Test	Dog	Cat	Horse	Cow	Units	Conversion factor	SI units
Alanine aminotransferase (ALT)	10–109	25–97	–	–	U/L	Same	
Albumin	2.3–3.1	2.8–3.9	2.6–4.1	2.5–3.8	g/dL	10	g/L
Albumin/globulin ratio (A/G)	0.6–1.1	0.6–1.1	0.6–1.4	0.6–0.9			
Alkaline phosphatase (ALP)	1–114	0–45	–	–	U/L	same	
Ammonia	19–120	0–90	–	–	µg/dL	0.5872	µmol/L
Amylase	226–1063	550–1458	–	–	U/L	same	
Anion gap	5–17	7–17	0–9	6–14			
Aspartate aminotransferase (AST)	13–15	7–38	160–412	60–125	U/L	same	
Bile acids (fasting)	0–8	0–5	0–20	–	µmol/L	same	
Bile acids (2-hour postprandial)	0–30	0–15	–	–			
Bilirubin, total	0.0–0.3	0.0–0.1	0.0–3.2	0.0–1.6	mg/dL	17.10	µmol/L
Bilirubin, direct (conjugated)	0.0–0.3	0.0–0.1	0.0–0.4	0.0–0.2	mg/dL	17.10	µmol/L
Blood gases							
pH	7.31–7.42	7.24–7.40	7.32–7.44	7.35–7.50			
HCO_3-	17–24	17–24	24–30	20–30	mEq/L	1.0	µmol/L
PCO_2	29–42	29–42	36–46	35–44	mmHg	0.1333	kPa
PO_2	85–95	85–95	94	92	mmHg	0.1333	kPa
Bromosulfophthalein (BSP) (small animals)	0–5	0–3	–	–	% retention		
Bromosulfophthalein (BSP) (large animals)	–	–	2.4–4.1	2.0–3.7	T1/2 (min)		
Cobalamin	200–400	–	–	–	ng/L	same	
Calcium	9.1–11.7	8.7–11.7	10.2–13.4	8.0–11.4	mg/dL	0.2495	mmol/L
Chloride	110–124	115–130	98–109	99–107	mEq/L	1.0	mmol/L
Cholesterol	135–278	71–156	–	–	mg/dL	0.02586	mmol/L
Cortisol, baseline	0.5–3.0	0.5–4.0	3.0–6.0	–	µg/dL	27.59	nmol/L
Creatine kinase (CK)	52–368	69–214	60–330	0–350	U/L	same	
Creatinine	0.5–1.7	0.9–2.2	0.4–2.2	0.5–2.2	mg/dL	88.40	µmol/L
Fibrinogen	150–300	150–300	100–400	100–600	mg/dL	0.01	g/L
Folate	4.8–13.0	–	–	–	µg/L	same	
Gamma glutamyltransferase (GGT)	–	–	6–32	6–17.4	U/L	same	

Free ebooks ==> www.Ebook777.com

Globulin, total	2.7–4.4	2.6–5.1	2.6–4.0	3.0–3.5	g/dL	10	g/L
α_1	0.2–0.5	0.3–0.9	0.1–0.7	0.7–0.9	g/dL	10	g/L
α_2	0.3–1.1	0.3–0.9	0.1–0.7	–	g/dL	10	g/L
β_1	0.6–1.2	0.4–0.9	0.4–1.6	0.8–1.2	g/dL	10	g/L
β_2	–	0.3–0.6	0.3–0.9	–	g/dL	10	g/L
γ	0.5–1.8	1.7–2.7	0.6–1.9	1.7–2.3	g/dL	10	g/L
Glucose	76–119	60–120	62–134	40–100	mg/dL	0.0555	mmol/L
Iron (serum, SI)	94–122	68–215	73–140	57–162	mg/dL	0.1791	µmol/L
Iron-binding capacity, total (TIBC)	165–418	173–420	273–402	120–348	mg/dL	0.1791	µmol/L
Lactic dehydrogenase (LDH)	0–236	58–120	112–456	–	U/L	same	
Lipase	60–330	0–76	–	–	U/L	same	
Magnesium	1.6–2.4	1.7–2.6	1.4–2.3	1.5–2.9	mg/dL	0.4114	mmol/L
Osmolality	288–305	280–305	270–300	270–300	mOsmol/kg	same	
Phosphorus	2.9–5.3	3.0–6.1	1.5–4.7	5.6–8.0	mg/dL	0.3229	mmol/L
Potassium	3.9–5.1	3.7–6.1	2.9–4.6	3.6–4.9	mEq/L	1.0	mmol/L
Protein, total (serum)	5.4–7.5	6.0–7.9	5.6–7.6	6.7–7.5	g/dL	10	g/L
Protein, total (plasma)	6.0–7.5	6.0–7.5	6.0–8.5	6.0–8.0	g/dL	10	g/L
Sodium	142–152	146–156	128–142	136–144	mEq/L	1.0	mmol/L
Sorbitol dehydrogenase (SDH)	–	–	1–8	4.3–15.3	U/L	same	
TCO2	14–26	13–21	22–33	22–34	mEq/L	1.0	mmol/L
Thyroxine (T4), baseline	1.5–4.0	0.8–4.0	1.0–4.0	–	mg/dL	12.87	nmol/L
Thyroid stimulating hormone (TSH)	0.02–0.32	–	–	–	ng/mL	1	µg/L
Triglycerides	40–169	27–94	6–54	–	mg/dL	0.0113	mmol/L
Trypsin-like immunoreactivity (TLI)	5.2–35	–	–	–	µg/L	same	
Urea nitrogen (BUN)	8–28	19–34	11–27	10–25	mg/dL	0.3570	mmol urea/L
Urine protein/urine creatinine ratio	<0.5	<0.5	–	–			

*These reference intervals are from the University of Georgia College of Veterinary Medicine and are to be used with the study cases in this textbook (unless otherwise indicated). These reference intervals are not appropriate for interpretation of data from other laboratories.

www.Ebook777.com

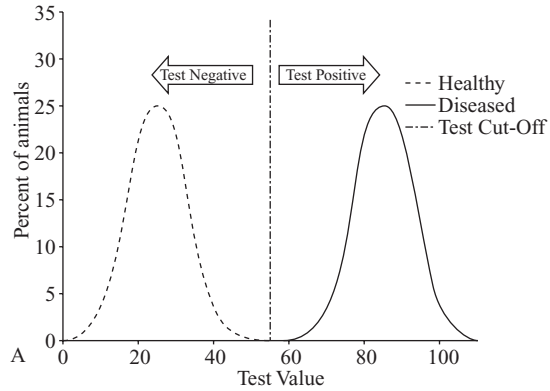


FIGURE 13.2a. Histogram of a theoretically perfect laboratory test, in which healthy and diseased animals have test values that do not overlap. In a perfect scenario, the cut-off differentiates between both groups; all animals that test negative are healthy and all animals that test positive are diseased.

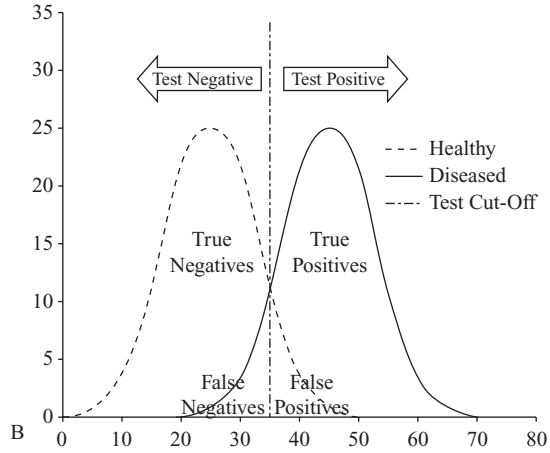


FIGURE 13.2b. Most tests have overlapping values for healthy and diseased populations of patients. With a central cut-off value, most healthy animals test negative and most diseased animals test positive, but some have false-positive or false-negative test results.

2. The choice of a cut-off depends on the purpose of the test and the consequences of the test result.

3. In an ideal test, the range of values in healthy animals does not overlap with the range of values in diseased animals (Figure 13.2a). All healthy animals test negative and all diseased animals test positive at the selected cut-off.

4. With most tests, the ranges of values in healthy and diseased animals overlap (Figure 13.2b). Therefore, as the cut-off changes, all other epidemiological variables change.

D. To quantify and characterize epidemiological variables at the selected cut-off, a 2 × 2 contingency table is used (Table 13.4).

1. In the table, the disease status is placed in columns and the test status is in rows.

a. The true disease status of animals is not based on the test being currently evaluated, but on another more definitive test called a gold standard. Common gold standards include biopsy, necropsy, PCR, etc.

Free ebooks ==> www.Ebook777.com

TABLE 13.4
EPIDEMIOLOGY 2 × 2 CONTINGENCY TABLE.

		Actual Disease Status	
		Positive (D+)	**Negative (D-)**
Test Result			
Positive (T+)		**a (TP)**	**b (FP)**
Negative (T-)		**c (FN)**	**d (TN)**
			n
Parameter		**Formula**	**Formula**
Disease prevalence		(TP+FN)/n	(a+c)/(a+b+c+d)
Sensitivity		TP/(TP+FN)	a/(a+c)
Specificity		TN/(TN+FP)	d/(b+d)
Positive likelihood ratio		TP/FP	a/c
Negative likelihood ratio		FN/TN	b/d
Odds ratio		(TP+FN)/(TN+FP)	(a+c)/(b+d)
Positive predictive value		TP/(TP+FP)	a/(a+b)
Negative predictive value		TN/(TN+FN)	d/(c+d)

b. Disease-positive patients (as determined by the gold standard) are placed in the left column.
c. Disease-negative patients (as determined by the gold standard) are placed in the right column.
d. Test values that fall above the selected cut-off are considered abnormal and called positive for the disease or condition. These are placed in the top row.
e. Test values that fall below the selected test cut-off are considered negative and are in the bottom row.

2. Each quadrant of the table represents a different group of patients classified by their true disease status and their test result.
a. Those in the top left quadrant (a) both have the disease and tested positive. These are the true-positives (TP).
b. Those in the bottom right quadrant (d) are both disease-free and tested negative. These are the true-negatives (TN).
c. Those in the top right quadrant (b) are disease-free but tested positive. These are the false-positives (FP).
d. Those in the bottom left quadrant (c) have the disease but tested negative. These are the false-negatives (FN).

E. Laboratory tests vary in their FP and FN rates for various diseases and are defined by their sensitivity, specificity, and likelihood ratios.

1. Sensitivity (Se) is a measure of the ability of the test to identify potentially diseased individuals. The higher the sensitivity, the more likely a diseased patient will test positive.
a. Sensitivity is defined as the frequency of a positive or abnormal test result when a disease is present (i.e., the percentage of diseased animals that test positive).
b. $\text{Sensitivity} = [\text{TP} \div (\text{TP} + \text{FN})] \times 100 = [\text{a} \div (\text{a} + \text{c})] \times 100$
c. A screening test is used primarily to detect the possible presence of a disease. High sensitivity is necessary for a screening test, because few diseased patients will incorrectly test negative. A positive result for a test with high sensitivity does not confirm the presence of the disease condition, but a negative result effectively eliminates that disease.

www.Ebook777.com

2. Specificity is the ability of a laboratory test to identify patients that do not have disease, or alternatively stated, to identify only patients with disease. The higher the specificity, the more likely a non-diseased patient will test negative.

a. Specificity is defined as the frequency of a negative or normal test result when disease is absent (i.e., the percentage of disease-free patients that test negative).

b. Specificity = $[TN \div (TN + FP)] \times 100 = [d \div (b + d)] \times 100$

c. A confirmatory or diagnostic test confirms the presence of disease, and is especially important when the therapy is potentially harmful to a non-diseased patient. High specificity is necessary for a confirmatory or diagnostic test because few non-diseased patients will incorrectly test positive. A positive result for a test with high specificity effectively rules in that condition, whereas a negative result does not confirm the absence of disease.

F. Laboratory tests are seldom highly sensitive and highly specific at the same time.

1. There is usually a trade-off between sensitivity and specificity; when sensitivity increases, specificity decreases. Therefore, the selection of a cut-off value varies with the purpose of the test and the consequences of a test result.

2. If the selected cut-off is decreased (Figure 13.2c), more animals test positive, the sensitivity of the test increases, and the specificity decreases. As a result, there are fewer false-negatives (FN), but more false-positives (FP). The lower the cut-off, the more likely a diseased patient will correctly test positive, but more non-diseased patients will incorrectly test positive.

3. If the selected cut-off is increased (Figure 13.2d), fewer animals test positive, the sensitivity decreases, and the specificity increases. As a result, there are fewer false-positives (FP), but more false-negatives (FN). The higher the cut-off, fewer non-diseased patients will incorrectly test positive, but more actually diseased patients will incorrectly test negative. Confirmatory or diagnostic tests tend to have high cut-offs.

4. In most cases, a screening test is followed by a confirmatory test, especially when treatment is potentially harmful.

G. The inverse relationship between sensitivity and specificity can be graphically represented with a receiver operator characteristic curve (ROC curve) as in Figure 13.3. The ROC curve can be useful to select a cut-off.

1. A ROC curve graphs the sensitivity (or true-positive rate) on the y-axis and 1-specificity (or false-positive rate) on the x-axis.

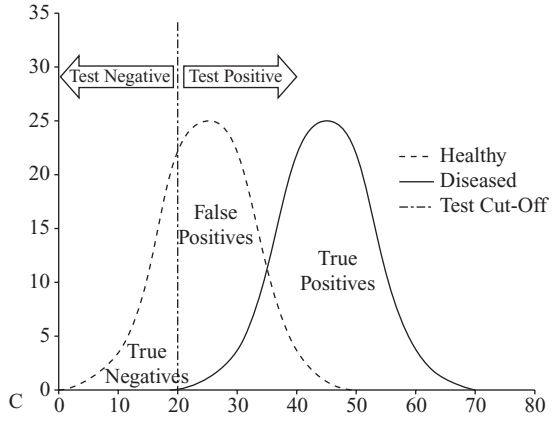


FIGURE 13.2c. If the test cut-off value is decreased, all diseased animals are detected. This is a good screening test. There are no false-negatives, but there are many false-positives. Not all animals that test positive are diseased, and a follow-up confirmatory or diagnostic test is necessary to identify the truly diseased animals.

Free ebooks ==> www.Ebook777.com

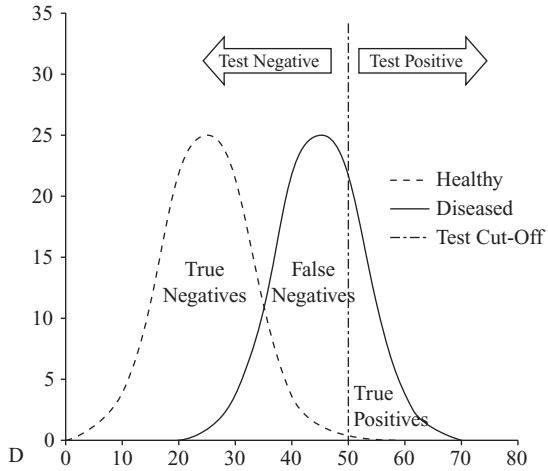


FIGURE 13.2d. If the cut-off value is increased, only diseased animals test positive. There are no false positives, but there are many false-negatives. This is a good confirmatory or diagnostic test. Not all animals that test negative are healthy, but all positives can be treated with confidence.

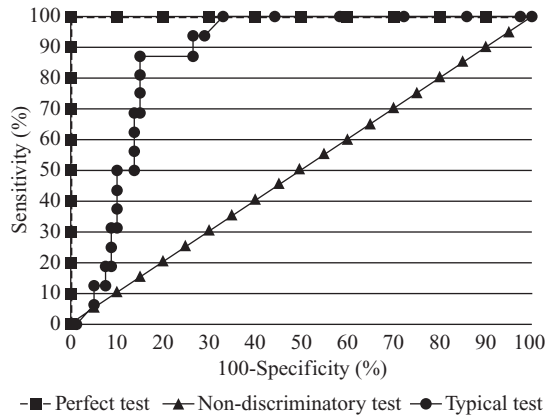


FIGURE 13.3. Example of a receiver operator characteristic (ROC) curve. To identify a good medical decision limit, the sensitivity is plotted against 1-specificity over a range of cut-offs for tests with continuous variables. Sensitivity and specificity are inversely related and vary depending on the selected cut-off. A perfect test contacts the top left corner, whereas a non-discriminatory test goes from the axis intersect to the top right corner. Most typical clinical tests approach but do not reach the top left corner.

2. The ROC curve of a perfect test with 100% sensitivity (no false-negatives) and 100% specificity (no false-positives) goes up the y-axis and then extends at a 90° angle parallel to the x-axis with a point in the upper left corner or coordinate (0,1).
3. The ROC curve of a useless test equivalent to chance (i.e., one with 50% sensitivity and 50% specificity) would create a diagonal line (or line of no-discrimination) from the bottom left to the top right corner.
4. Most laboratory tests yield an ROC curve that approaches the top left corner.
5. If the consequences of a false-positive or false-negative test result are equal for the patient, the best cut-off value is one with the greatest area under the curve. However, usually the cut-off is lowered when used as a screening test, or increased when used as a diagnostic test.

www.Ebook777.com

H. Likelihood ratios are a useful measure of a test. They indicate the probability of disease actually being present if the test is positive or of actually being absent if the test is negative. They are independent of the true prevalence and are based only on the test sensitivity and specificity at the chosen cut-off.

1. The positive likelihood ratio (LR+) indicates the power of a positive test and ranges from 1 to infinity. It is the likelihood of ruling in disease with a positive test. The higher the LR+, the more useful the test is in confirming disease. It is not the likelihood of the animal having disease.

a. The LR+ quantifies how much more likely the patient is to have the disease if the test is positive. A good confirmatory or diagnostic test has a high LR+.

b. $\text{LR+} = [\text{sensitivity} \div (1 - \text{specificity})] \times 100$.

2. The negative likelihood ratio (LR-) indicates the power of a negative test and ranges from 0 to 1. It is the likelihood of ruling out disease with a negative test. The lower the LR-, the more useful it is to confirm a negative disease status. It is not the likelihood of the animal being disease-free.

a. The LR- quantifies how much less likely the patient is to be have disease if the test is negative. A good screening test has a low LR-.

b. $\text{LR}- = [(1 - \text{sensitivity}) \div (\text{specificity})] \times 100$.

I. Sensitivity, specificity, and likelihood ratios are inherent to the test and fixed. However, the interpretation of a test result for an individual patient varies with the pre-test probability of disease or disease prevalence. The most common measures are odds ratio, positive predictive value, and negative predictive value.

1. Odds ratio. The probability of disease can be expressed as an odds ratio (OR). The OR is the proportion of diseased to non-diseased animals.

a. $\text{OR} = (\text{TP} + \text{FN}) \div (\text{TN} + \text{FP}) = (a + c) \div (b + d)$.

b. The OR changes with the prevalence of disease in the group. This varies with patient parameters such as signalment, history, and clinical signs. For example, the OR for heart disease is higher if the patient has a heart murmur because many patients with heart murmurs have heart disease. Conversely, the OR for ruptured cruciate ligament among patients with heart murmurs is low because heart murmurs are uncommon in animals with ruptured cruciates (heart murmurs are more common in small dogs and dogs with heart disease, whereas cruciate ruptures are more common in young, large-breed dogs, which do not often have heart murmurs).

c. The post-test odds are calculated as the pre-test odds multiplied by the test's likelihood ratio (see section H, above).

d. If the pre-test odds are different from the post-test odds, the test selection is appropriate. If the pre- and post-test odds are similar, the test does not provide any useful information.

2. Positive predictive value. The positive predictive value (PPV) is an indicator of the probability of having the disease if the patient tests positive. A test with a high positive predictive value is one in which most positive patients have disease.

a. PPV is defined as the percentage of diseased patients among patients that test positive.

b. $\text{PPV} = [\text{TP} \div (\text{TP} + \text{FP})] \times 100 = [a \div (a + b)] \times 100$.

3. Negative predictive value. The negative predictive value (NPV) is an indicator of the probability of being disease free if the patient tests negative. A test with a high negative predictive value is in which most negative patients are disease free.

a. NPV is defined as the percentage of non-diseased patients among patients that test negative.

b. $\text{NPV} = [\text{TN} \div (\text{TN} + \text{FN})] \times 100 = [d \div (c + d)] \times 100$.

4. Predictive values highly depend on the actual, but often unknowable, true disease prevalence.

a. As prevalence increases, the positive predictive value (PPV) and false-negatives (FN) increase. The more common a disease, the more likely a patient is to have the disease, regardless of the test result. A positive test is more likely to be correct if the prevalence is high, for example, if a test is used for animals with clinical signs suggestive of the disease.

Free ebooks ==> www.Ebook777.com

b. The negative predictive value and false-positive rate both increase as prevalence decreases. The rarer the disease, the more likely the patient is non-diseased, regardless of the test result. A negative test is more likely to be correct if the prevalence is already low.

c. When a disease is common, false-negatives are reduced by using a test with high sensitivity, though false-positives will occur.

d. When a disease is uncommon, false-positives are reduced with a highly specific test. Because the disease is rare, false-negatives are rare and not a concern.

5. If a laboratory screening test is useful, its sensitivity is high and PPV will be much greater than the disease prevalence. A good diagnostic test has a high NPV because it is specific, but may be a poor screening test when the prevalence of the disease is low.

6. The use of multiple tests changes the predictive values.

a. Performing laboratory tests in series (e.g., a second laboratory test is done if the first laboratory test is positive; the algorithmic approach) increases the PPV. Patients that test positive to a series of tests are more likely to be truly diseased. Test specificity is increased at the expense of test sensitivity when compared to using only a single test. Series of tests are used when a high degree of certainty is required in a diagnosis, such as when the treatment could harm a non-diseased patient.

b. Performing multiple laboratory tests in parallel (e.g., biochemical profiles or batteries of tests) increases the NPV. Test sensitivity is increased at the expense of test specificity. Patients with disease are unlikely to be missed when compared to using only a single test. The greater the number of tests in the profile, the greater the chance for FP test results. Biochemical profiles are best used to exclude diseases with a high degree of confidence. Thus, they are used as screening tests.

REFERENCES

Drobatz KJ: 2009. Measures of accuracy and performance of diagnostic tests. *J Vet Cardiol* 11 Suppl 1:S33–40.

Farver TB: 1997. Concepts of Normality in Clinical Biochemistry. *In:* Kaneko JJ, Harvey JW, Bruss M (eds): Clinical Biochemistry of Domestic Animals, 5th ed. Academic Press, San Diego.

Gardner IA, Greiner M: 2006. Receiver-operating characteristic curves and likelihood ratios: Improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. *Vet Clin Pathol* 35:8–17.

Harvey JW, Pate MG, Kivipelto J, et al: 2005. Clinical biochemistry of pregnant and nursing mares. *Vet Clin Pathol* 34:248–254.

International Federation of Clinical Chemistry, Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values (EPTRV): 1984. The theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clin Chim Acta* 137:97F-114F.

Jensen AL: 2000. Validation of Diagnostic Tests in Hematology Laboratories. *In:* Feldman BV, Zinkl JG, Jain NC, Schalm OW (eds): *Schalm's Veterinary* Hematology, 5th ed. Lippincott Williams and Wilkins, Philadelphia.

Kramer JW, Hoffmann WE: 1997. Clinical Enzymology. *In:* Kaneko JJ, Harvey JW, Bruss M (eds): Clinical Biochemistry of Domestic Animals, 5th ed. Academic Press, San Diego.

Lumsden JH: 2000. Quality Control. *In:* Feldman BV, Zinkl JG, Jain NC, Schalm OW (eds): *Schalm's Veterinary* Hematology, 5th ed. Lippincott Williams and Wilkins, Philadelphia, PA.

Lumsden JH: 2000. Reference Values. *In:* Feldman BV, Zinkl JG, Jain NC, Schalm OW (eds): *Schalm's Veterinary* Hematology, 5th ed. Lippincott Williams and Wilkins, Philadelphia.

www.Ebook777.com

Mahaffey EA: 2003. Quality Control, Test Validity, and Reference Values. *In:* Latimer KS, Mahaffey EA, Prasse KW (eds): Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 4th ed. Iowa State University Press, Ames, IA.

Solberg HE: 1993. A guide to IFCC recommendations on reference values. *J Int Fed Clin Chem* 5:162–165.

Solberg HE: 1999. Establishment and Use of Reference Values. *In:* Burtis CA, Ashwood ER, Tietz NW (eds): Tietz Textbook of Clinical Chemistry, 3rd ed. W.B. Saunders, Philadelphia.

Westgard JO: 1999. Quality Management. *In:* Burtis CA, Ashwood ER, Tietz NW (eds): Tietz Textbook of Clinical Chemistry, 3rd ed. W.B. Saunders, Philadelphia.

Young DS, Bermes EW: 1999. Specimen Collection and Processing: Sources of Biological Variation. *In:* Burtis CA, Ashwood ER, Tietz NW (eds): Tietz Textbook of Clinical Chemistry, 3rd ed. W.B. Saunders, Philadelphia.

Free ebooks ==> www.Ebook777.com

CASE STUDIES

The following case studies have been designed to give the reader an opportunity to practice the interpretation of laboratory data, using the principles and concepts presented in the various chapters of this textbook. When reading a particular chapter of the textbook, the reader is referred to these illustrative cases by number. Each case study includes signalment and presenting complaint(s). To rapidly discern abnormalities in the laboratory data, an H (high or above the reference interval) or L (low or below the reference interval) is placed adjacent to the abnormal test value. These data have been largely derived from actual clinical cases; however, minor alterations or additions have been made to the data sets to simplify or clarify the points being illustrated by each case. Therefore, these study cases should be used only to practice laboratory data interpretation and should not be referenced as actual clinical cases. Tables 13.1, 13.2, and 13.3 contain the reference intervals that have been derived for use at the University of Georgia College of Veterinary Medicine; they may not be appropriate to identify test abnormalities in data sets from other clinical laboratories (see Chapter 13). The reference interval tables should be used for interpretation of the laboratory data in these study cases, unless otherwise indicated.

CASE	DISEASE	SPECIES
1	Hemorrhagic anemia (ancylostomiasis)	dog
2	Autoimmune hemolytic anemia and thrombocytopenia	dog
3	Acute intravascular hemolytic anemia (red maple toxicosis)	horse
4	Estrogen-induced pancytopenia (Sertoli cell tumor)	dog
5	Chronic hemorrhagic anemia (iron-lack anemia)	dog
6	Acute salmonellosis	horse
7	Feline infectious peritonitis	cat
8	Pyometra	dog
9	Feline infectious panleukopenia (parvovirus infection)	cat
10	Multicentric lymphoma	dog
11	Renal abscess and secondary disseminated intravascular coagulation	dog
12	Hepatic encephalopathy (hepatic fibrosis)	horse
13	Portosystemic venous shunt with hepatic atrophy	dog
14	Acute pancreatic necrosis	dog
15	Exocrine pancreatic insufficiency, diabetes mellitus, and chronic renal failure	dog
16	Inflammatory bowel disease (malabsorption and protein-losing enteropathy)	dog
17	Muscle disease, myoglobinuric nephrosis	horse
18	Acute renal failure (ethylene glycol toxicosis)	dog

www.Ebook777.com

CASE	DISEASE	SPECIES
19	Renal amyloidosis (nephrotic syndrome)	dog
20	Feline lower urinary tract disease (feline urologic syndrome)	cat
21	Acute septic mastitis	cow
22	Nephrosis and perirenal hemorrhage	cow
23	Right abomasal displacement	cow
24	Acute pulmonary hemorrhage and edema (acute Paraquat® toxicosis)	dog
25	Rodenticide (coumarin) toxicosis	dog
26	Hyperadrenocorticism	dog
27	Hyperthyroidism	cat
28	von Willebrand's disease	dog
29	Urea toxicosis	cow
30	End stage renal disease with uremic pneumonitis	dog
31	Early primary hypothyroidism	dog
32	Possible glucocorticoid suppression of thyroid function	dog
33	Primary hypothyroidism (and phenobarbital administration)	dog
34	Hyperadrenocorticism and diabetes mellitus with ketoacidosis, hyperosmolality, necrotizing pancreatitis, and urinary tract infection	dog
35	Artifacts of lipemia, hemolysis, and improper sample handling	dog

Free ebooks ==> www.Ebook777.com

CASE 1 HEMORRHAGIC ANEMIA (ANCYLOSTOMIASIS)

SIGNALMENT: Canine, Pointer, male, 8 weeks old

PRESENTING PROBLEMS: Melena and mucous membrane pallor for several days

LABORATORY DATA:

Hematology

Hct	13	L	%
Hb	3.9	L	g/dL
RBC	1.59	L	$\times 10^6/\mu L$
MCV	81	H	fL
MCH	24.5		pg
MCHC	30.0	L	%
Retic	16.6	H	%
Abs retic	264	H	$\times 10^3/\mu L$
NRBC	3	H	/100 WBC

RBC morphology: anisocytosis, polychromasia

Platelets	653	H	$\times 10^3/\mu L$
WBC	17.5	H	$\times 10^3/\mu L$
Seg	13.650 (78%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	2.625 (15%)		$\times 10^3/\mu L$
Mono	0.525 (3%)		$\times 10^3/\mu L$
Eos	0.700 (4%)		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$

WBC morphology: normal

Serum Chemistry

Total protein	3.8	L	g/dL
Albumin	1.8	L	g/dL
A/G ratio	0.90		

Other Tests

Fecal: *Ancylostoma* sp. ova

PROBLEMS:

1. **Hemorrhagic anemia**.
   a. **Regenerative anemia** accompanied by **hypoproteinemia**, an **A/G ratio within the reference interval** (panhypoproteinemia), and clinical signs of hemorrhage indicate blood loss.
   b. **Hypoalbuminemia** and calculated **hypoglobulinemia** are present. Because all components of plasma are lost during external hemorrhage, the **A/G ratio** remains **within the reference interval**.
   c. The **increased MCV** (**macrocytosis**) and low MCHC (**hypochromia**) are due to reticulocytosis. Reticulocytes are large, young, anucleate erythrocytes that lack their full concentration of hemoglobin.
   d. Metarubricyte release may accompany intense erythrocytic regeneration.

2. **Thrombocytosis**. Thrombocytosis is frequently observed in chronic hemorrhage, particularly with ancylostomiasis. The mechanisms of thrombocytosis may include mobilization of splenic platelet reserves and increased thrombocytopoiesis following partial loss of total platelet mass via hemorrhage.

3. **Neutrophilic leukocytosis**. A **mature neutrophilia** frequently is associated with hemorrhage. In this puppy, neutrophilia may have resulted from a combination of hemorrhage (the precise molecular mechanism responsible for neutrophilia in this instance is poorly understood) and release of chemotactic factors at the sites of mucosal damage by hookworms.

SUMMARY: The dog responded to a blood transfusion and administration of anthelmintics.

www.Ebook777.com

CASE 2 AUTOIMMUNE HEMOLYTIC ANEMIA AND THROMBOCYTOPENIA

SIGNALMENT: Canine, Old English Sheepdog, male, 2 years old

MEDICAL HISTORY: Previous corticosteroid administration

PRESENTING PROBLEMS: Lethargy, weakness, pale mucous membranes, icterus

LABORATORY DATA:

	Hematology		
Hct	15.0	L	%
Hb	4.4	L	g/dL
RBC	1.79	L	$\times 10^6/\mu L$
MCV	84	H	fl
MCH	24.6		pg
MCHC	29.3	L	%
Retic	24	H	%
Abs retic	430	H	$\times 10^3/\mu L$
NRBC	6	H	/100 WBC
RBC morphology: anisocytosis, polychromasia, spherocytes			
Platelets	88	L	$\times 10^3/\mu L$
MPV	15	H	fL
WBC	44.7	H	$\times 10^3/\mu L$
Seg	35.760 (80%)	H	$\times 10^3/\mu L$
Band	4.470 (10%)	H	$\times 10^3/\mu L$
Lymph	0.447 (1%)	L	$\times 10^3/\mu L$
Mono	4.023 (9%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Other Tests		
Direct antiglobulin (Coombs') test	positive		
Rocky Mt spotted fever titer	negative		
Ehrlichia spp. titers	negative		

Free ebooks ==> www.Ebook777.com

	Serum Chemistry		
BUN	18		mg/dL
Creatinine	0.5		mg/dL
Total protein	6.5		g/dL
Albumin	2.9		g/dL
A/G ratio	0.81		
ALP	625	H	U/L
ALP (post-levam) 575 H U/L			
Levamisole resistance	92		%
ALT	536	H	U/L
Glucose	93		mg/dl
Sodium	146		mmol/L
Potassium	4.6		mmol/L
Chloride	115		mmol/L
Total CO_2	20		mmol/L
Anion gap	15.6		mmol/L
Calcium	9.9		mg/dL
Total bilirubin	9.8	H	mg/dL
Conj. bilirubin	4.3	H	mg/dL
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	hazy		
Sp Gr	1.007		
pH	7.5		
Protein	negative		
Glucose	negative		
Ketone	negative		
Bilirubin	2+		
Blood	negative		
Sediment	fat droplets		

PROBLEMS:

1. **Macrocytic, hypochromic, regenerative anemia (immune-mediated hemolytic anemia).**
   a. **Regenerative anemia** with icterus, **hyperbilirubinemia**, and a predominance of **unconjugated (indirect) bilirubin** indicates hemolysis.
   b. **Extravascular hemolysis** is present; intravascular hemolysis is excluded based on the absence of hemoglobinemia and hemoglobinuria (negative occult blood test on the urine specimen).
   c. **Antibody-mediated extravascular hemolysis** is supported by **spherocytosis** and a **positive direct antiglobulin (Coombs') test**.
   d. **Reticulocytosis** of this magnitude also suggests hemolysis because iron from lysed erythrocytes is readily reused. In contrast, iron is lost from the body in hemorrhagic anemia and mobilization of storage iron is accomplished more slowly; reticulocytosis is of lower magnitude.
   e. The anemia is **macrocytic** because of numerous reticulocytes, which are larger cells. Reticulocytes lack their full component of hemoglobin; therefore, the anemia appears **hypochromic**.
   f. **Metarubricytosis** (**nRBCs**) may accompany intense erythrocytic regeneration or may be prematurely released from the bone marrow following anemia-induced hypoxia.

www.Ebook777.com

2. **Neutrophilic leukocytosis with a left shift, monocytosis, and lymphopenia**. These changes in the leukogram can be attributed to both hemolysis and corticosteroids. Extravascular hemolysis is characterized by neutrophilia. A left shift may occur, particularly when the hemolysis is immune-mediated. Exogenous corticosteroid administration or endogenous cortisol release (stress) can cause neutrophilia (usually without a left shift), lymphopenia, and monocytosis in the dog. The bone marrow does not contain a storage pool of monocytes; these cells are released into the blood at a relatively young age.

3. **Thrombocytopenia**. The cause is most likely extramarrow destruction because the **increased MPV** suggests increased turnover of platelets; younger platelets have a larger MPV. Immune-mediated destruction of platelets is the most likely mechanism because other common causes of thrombocytopenia such as Rocky Mountain spotted fever and ehrlichiosis have been eliminated by serologic tests. The blood smear also was evaluated for platelet aggregation secondary to poor venipuncture, but platelet aggregates were not present. Immune-mediated anemia and immune-mediated thrombocytopenia can occur concurrently. This condition is called Evan's syndrome.

4. **Hepatocellular injury**. Anemia-induced hypoxic injury to centrilobular hepatocytes can cause enzyme leakage (**increased ALT activity**) with minimal cholestasis. Cholestasis induces increased activity of the levamisole-sensitive, hepatic isoenzyme of ALP, which is minimal in this case. ALT activity also may be induced with corticosteroid-induced glycogen accumulation in hepatocytes (steroid hepatopathy).

5. **Increased ALP activity**. ALP activity that is resistant to levamisole treatment is due to the steroidal isoenzyme. This dog probably had been treated with corticosteroids prior to presentation for clinical signs suggestive of anemia and /or liver disease.

6. **Bilirubinuria**. The urine test for bilirubin detects conjugated (direct) bilirubin. Bilirubinuria precedes the development of hyperbilirubinemia. Conjugated bilirubin is water soluble and is rapidly filtered into the urine by the kidneys.

SUMMARY: The dog responded to the corticosteroid therapy with an increasing hematocrit.

Free ebooks ==> www.Ebook777.com

CASE 3 ACUTE INTRAVASCULAR HEMOLYTIC ANEMIA (RED MAPLE TOXICOSIS)

SIGNALMENT: Equine, Appaloosa, gelding (castrated male), 12 years old

HISTORY: Observed eating leaves from a red maple tree that had fallen from the fence line into the pasture during a thunderstorm

PRESENTING PROBLEMS: Depression, dehydration, icterus, chocolate-colored blood, dark brown urine, orange-red plasma

LABORATORY DATA:

	Hematology		
Hct	25.2	L	%
Hb	11.2		g/dl
RBC	5.50	L	$\times 10^6/\mu L$
MCV	45		fL
MCH	20	H	pg
MCHC	44	H	%
RBC morphology: Heinz bodies, eccentrocytes			
Platelets: adequate			
WBC	14.8	H	$\times 10^3/\mu L$
Seg	13.764 (93%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	0.592 (4%)	L	$\times 10^3/\mu L$
Mono	0.444 (3%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
Fibrinogen	500	H	mg/dL
	Serum Chemistry		
Creatinine	5.6	H	mg/dL
Total protein	8.5	H	g/dL
Albumin	3.9		g/dL
A/G ratio	0.85		
AST	518	H	U/L
T. Bilirubin	11.6	H	mg/dL
D. Bilirubin	1.8	H	mg/dL
Methemoglobin	208	H	µmol/L

continued

www.Ebook777.com

	Urinalysis
Urine source	voided
Color	brown
Turbidity	cloudy
Sp Gr	1.015
pH	8.0
Protein	3+
Glucose	negative
Ketone	negative
Bilirubin	1+
Blood	4+
Sediment	$CaCO_3$ crystals, mucus

PROBLEMS:

1. Hemolytic anemia. **Anemia** associated with **hyperbilirubinemia**, **hemoglobinemia**, and **hemoglobinuria** indicates **intravascular hemolysis**. Red plasma and an **increased MCHC** indicate free hemoglobin or **hemoglobinemia**. **Heinz bodies** and **eccentrocytes** indicate oxidative damage to erythrocytes from ingestion of red maple leaves. Heinz bodies can interfere with measurement of the hemoglobin, which will alter both the MCH and MCHC. In contrast to other animals, horses do not exhibit reticulocytosis during anemia. Therefore, all equine anemias appear nonregenerative.

2. Methemoglobinemia. Methemoglobin formation occurs when heme iron is oxidized from the ferrous (2^+) to the ferric (3^+) form, presumably by the same oxidant that denatures the globin chains forming Heinz bodies. In health, methemoglobin is constantly being formed, but it is reduced to hemoglobin by the methemoglobin reductase pathway; therefore, <1% of the total hemoglobin is in the methemoglobin form. Marked oxidant exposure may promote the formation of methemoglobin. The blood and mucous membranes may appear brown when >10% of the total hemoglobin has been converted to methemoglobin. Methemoglobin values >60% to 65% of the total hemoglobin are incompatible with life. Methemoglobinemia may or may not accompany oxidative hemolysis.

3. Neutrophilic leukocytosis and lymphopenia. Neutrophilia is observed commonly in hemolytic anemias. Destruction of erythrocytes, like destruction of other tissues, may incite a tissue demand for neutrophils. Endogenous cortisol release also may contribute to the neutrophilia and cause lymphopenia. Lymphopenia probably has resulted from temporary redistribution of recirculating lymphocytes.

4. Hyperfibrinogenemia. Fibrinogen is an acute phase reactant whose concentration increases during inflammation. Hyperfibrinogenemia may be the earliest indicator of inflammation and may precede changes in the leukogram of horses.

5. Azotemia. Azotemia (increased creatinine concentration) may be of prerenal, renal, or post-renal origin. Because the specimen is voided, post-renal azotemia is excluded. Dehydration may contribute to prerenal azotemia because the glomerular filtration rate decreases. Renal azotemia from **renal failure** also is an important consideration. The **low urine Sp Gr urine** suggests renal involvement. Following intravascular hemolysis, hemoglobinuria can lead to renal tubular degeneration and necrosis (hemoglobinuric nephrosis).

6. Hyperproteinemia. Hyperproteinemia with a **normal A/G ratio** in a dehydrated animal suggests a relative increase in protein concentration. Hemoglobinemia also may have contributed to the hyperproteinemia.

Free ebooks ==> www.Ebook777.com

7. **Increased AST activity.** AST activity is present in many cells including hepatocytes, myocytes, and erythrocytes. Increased AST activity in the serum of this horse is probably secondary to intravascular hemolysis; however, liver and muscle are significant sources of enzymatic activity in disease. Evaluation of SDH and CK activities could exclude or confirm hepatic and muscle damage, respectively.

8. **Unconjugated hyperbilirubinemia**. Hyperbilirubinemia with a preponderance of **unconjugated (indirect) bilirubin** in most species suggests hemolysis to the extent that the hepatic uptake and conjugation is overwhelmed. However, in the horse, unconjugated bilirubin predominates in all types of icterus. Hyperbilirubinemia also may accompany anorexia in horses.

9. **Brown urine with low specific gravity, proteinuria, bilirubinuria, and positive occult blood test. Brown discoloration** of the urine can occur with hematuria, hemoglobinuria, and myoglobinuria. The presence of hemoglobinemia, positive occult blood test, and lack of erythrocytes in the urinary sediment suggest **hemoglobinuria**. Hemoglobinuria may be confirmed by ammonium sulfate precipitation of hemoglobin from the urine. The low urine specific gravity could indicate impending renal failure, but the specific gravity is highly variable on a single, free-catch urine specimen. The reagent strip protein detection pad will change color in the presence of an alkaline pH. Because the pH of the urine is alkaline, an acid precipitation test should be done to confirm proteinuria. The source of protein is probably filtered hemoglobin, but glomerular or tubular lesions also are possible, especially if hemoglobinuric nephrosis is present. Although unconjugated hyperbilirubinemia is more common in the horse, some of the excess bilirubin may be conjugated. Bilirubin in the urine is conjugated, water soluble, and rapidly filtered from the plasma by the glomerulus. Bilirubinuria precedes detectable hyperbilirubinemia.

SUMMARY: Hemoglobinuric nephrosis secondary to red maple leaf toxicosis was determined as the cause of death at necropsy.

www.Ebook777.com

CASE 4 ESTROGEN-INDUCED PANCYTOPENIA (SERTOLI CELL TUMOR)

SIGNALMENT: Canine, American Eskimo Dog, castrated male, 10 years old

PRESENTING PROBLEMS: Bilateral cryptorchid, enlarged prostate, frequent attempts to urinate, dribbling urine

LABORATORY DATA:

	Hematology		
Hct	15.1	L	%
Hb	5.3	L	g/dL
RBC	2.21	L	$\times 10^6/\mu L$
MCV	68		fL
MCH	24.0		pg
MCHC	35.1		%
Retic	0.3		%
Abs retic	6.63	L	$\times 10^3/\mu L$
RBC morphology: normal			
Platelets	6	L	$\times 10^3/\mu L$
MPV	7.5		fL
WBC	1.6	L	$\times 10^3/\mu L$
Seg	0.464 (29%)	L	$\times 10^3/\mu L$
Band	0.176 (11%)		$\times 10^3/\mu L$
Lymph	0.928 (58%)		$\times 10^3/\mu L$
Mono	0.016 (1%)		$\times 10^3/\mu L$
Eos	0.016 (1%)		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	catheterized		
Color	yellow		
Turbidity	cloudy		
Sp Gr	1.035		
pH	8.0		
Protein	1+		

Free ebooks ==> www.Ebook777.com

Glucose	negative
Ketone	negative
Bilirubin	1+
Blood	negative
Sediment	0–2 RBCs/hpf innumerable WBCs/hpf many squamous cells

Other Tests

Bone marrow aspirate: small, fatty, hypocellular particles composed mostly of stromal cells

Opinion: aplastic anemia

PROBLEMS:

1. **Aplastic anemia (pancytopenia). Nonregenerative anemia** with normal erythrocytic indices, **neutropenia**, and **thrombocytopenia** indicate **aplastic anemia** and suggest a multipotential stem cell disorder. Cytologic evaluation of the bone marrow confirmed **bone marrow aplasia**. Neutropenia from decreased cellular production usually has a minimal left shift. The **left shift** in this dog probably indicates an increased tissue demand for neutrophils (prostatic inflammation) as well as depletion of bone marrow storage reserves of mature neutrophils (segmenters). Bone marrow examination also revealed a lack of megakaryocytes and failure of thrombocytopoiesis. The MPV is within the reference interval, suggesting that excessive platelet turnover is not occurring. Estrogen toxicosis is a cause of myelosuppresion in the dog. This condition also may result from a paraneoplastic syndrome (Sertoli cell tumor that produces estrogen) or administration of an estrogen-based drug.

2. **Urinary tract inflammation, possible prostatitis, and pyuria** in a catheterized sample suggests urinary tract inflammation. **Squamous epithelial cells** in the catheterized urine specimen of this dog suggest squamous metaplasia of the prostatic epithelium secondary to estrogens. The alkaline pH of the urine will cause the protein pad of the reagent strip to change color nonspecifically. Therefore, proteinuria must be confirmed by another test such as acid precipitation (Robert's test). Mild **proteinuria** associated with leukocytes in the urine, in the absence of occult blood and erythrocytes, suggests **urinary tract inflammation**. Further diagnostic testing is indicated to confirm or exclude the possibilities of prostatitis and infection.

SUMMARY: Exploratory laparotomy revealed a unilateral testicular mass. Castration was performed and the prostate gland was biopsied. Histologically, the testicular mass was diagnosed as a Sertoli cell tumor. The prostatic biopsy revealed squamous metaplasia of the prostatic epithelium and purulent inflammation. Estrogen secretion by the tumor (paraneoplastic syndrome) was responsible for the generalized myelosuppression and aplastic anemia as well as prostatic squamous metaplasia. The purulent prostatitis occurred secondary to the squamous metaplasia. Neutropenia may predispose this dog to infection.

www.Ebook777.com

CASE 5 CHRONIC HEMORRHAGIC ANEMIA (IRON-LACK ANEMIA)

SIGNALMENT: Canine, mixed breed, spayed female, 5 years old

PRESENTING PROBLEMS: Lethargy, weakness, pale mucous membranes, melena (dark-colored feces)

LABORATORY DATA:

	Hematology		
Hct	12.8	L	%
Hb	4.0	L	g/dL
RBC	2.25	L	$\times 10^6/\mu L$
MCV	56.9	L	fL
MCH	17.8	L	pg
MCHC	31.3	L	%
Retic	3.6	H	%
Abs retic	81	H	$\times 10^3/\mu L$
NRBC	2	H	/100 WBC
RBC morphology: anisocytosis, polychromasia			
Platelets	771	H	$\times 10^3/\mu L$
WBC	40.1	H	$\times 10^3/\mu L$
Seg	35.689 (89%)	H	$\times 10^3/\mu L$
Band	0.401 (1%)	H	$\times 10^3/\mu L$
Lymph	1.203 (3%)		$\times 10^3/\mu L$
Mono	2.807 (7%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	catheterized		
Color	straw		
Turbidity	clear		
Sp Gr	1.036		
pH	7.5		
Protein	negative		
Glucose	negative		
Ketone	negative		
Bilirubin	negative		
Blood	negative		
Sediment	amorphous crystals		

Free ebooks ==> www.Ebook777.com

	Serum Chemistry		
BUN	15		mg/dL
Creatinine	0.9		mg/dL
Total protein	6.5		g/dL
Albumin	2.5		g/dL
A/G ratio	0.63		
ALP	64		U/L
ALT	214	H	U/L
Glucose	115		mg/dL
Sodium	147		mmol/L
Potassium	4.3		mmol/L
Chloride	125		mmol/L
Total CO_2	11		mmol/L
Anion gap	11		mmol/L
Calcium	9.5		mg/dL
	Other Tests		
Serum iron	42	L	µg/dL
Total iron binding capacity (TIBC)	380		µg/dL
% saturation	11.1	L	%
Fecal occult blood			positive

Bone marrow aspirate:
hypercellular particles
increased megakaryocytes
high-normal M/E ratio
increased late rubricytes and metarubricytes absence of macrophage iron

Opinion:
erythroid hyperplasia and delayed maturation
granulocytic hyperplasia
megakaryocytic hyperplasia iron lack

PROBLEMS:

1. **Iron-lack anemia**. A **microcytic**, **hypochromic**, **regenerative anemia** suggests early iron-lack anemia from blood loss. With continued iron loss, the anemia will eventually become nonregenerative. The **lack of stainable macrophage iron**, **low serum iron concentration**, and **decreased saturation of transferrin** confirm iron deficiency. Serum ferritin concentration provides a more accurate assessment of total body iron status, but the assay is species-specific.

2. **Neutrophilic leukocytosis**. Neutrophilia occurs commonly with regenerative anemia, which may result in increased production of all cell lines within the bone marrow. Although a clinically important left shift has not yet occurred, sequential leukograms should be considered to detect a developing left shift and identify early infection.

3. **Thrombocytosis**. Increased platelet production commonly accompanies hemorrhagic anemia.

4. **Hepatocellular injury**. Mildly **increased ALT activity** is probably the result of hypoxia-induced enzymatic leakage from centrilobular hepatocytes. In the hepatic lobules, oxygenated blood flows from the portal triads to the central veins. Therefore, the centrilobular hepatocytes are more susceptible to hypoxia.

www.Ebook777.com

5. **Normoproteinemia and normoalbuminemia**. Hemorrhagic anemias often have hypoproteinemia with a normal A/G ratio because albumin and globulin are lost concurrently. This patient may have been dewormed prior to presentation, in which case the protein concentration will normalize faster than changes in the erythroid parameters. Alternatively, the patient may be slightly hypoproteinemic and hypoalbuminemic but dehydrated, elevating the protein and albumin concentrations into the reference interval.

6. **Bone marrow hyperplasia**. An increase in the granulocytic series of the marrow (**high M/E ratio**) is expected with neutrophilia. **Megakaryocytic hyperplasia** may occur with thrombocytosis, unless the platelets are being mobilized from splenic reserves. With iron deficiency, a critical intracellular concentration of hemoglobin necessary to stop cell division is not reached. Therefore, an extracellular division occurs, causing microcytosis and increasing the number of late rubricytes and metarubricytes in the bone marrow. Ineffective erythropoiesis accompanies the iron depletion.

SUMMARY: A bleeding intestinal mass was observed during surgery. The mass was resected and an anastomosis was performed. The histological diagnosis of the mass was intestinal leiomyosarcoma with hemorrhage.

Free ebooks ==> www.Ebook777.com

CASE 6 ACUTE SALMONELLOSIS

SIGNALMENT: Equine, Thoroughbred, gelding (castrated male), 10 years old

PRESENTING PROBLEMS: Diarrhea of 24 hours duration, anorexia, weakness, pyrexia, dehydration estimated to be 7%, hyperpnea

LABORATORY DATA:

	Hematology		
Hct	51.5	H	%
Hb	17.1	H	g/dL
RBC morphology: normal			
Platelets: adequate			
WBC	4.2	L	$\times 10^3/\mu L$
Seg	0.882 (21%)	L	$\times 10^3/\mu L$
Band	2.310 (55%)	H	$\times 10^3/\mu L$
Lymph	0.126 (3%)	L	$\times 10^3/\mu L$
Mono	0.882 (21%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: cytoplasmic basophilia and vacuolation			
Fibrinogen	1100	H	mg/dL
	Serum Chemistry		
BUN	51	H	mg/dL
Total protein	7.8	H	g/dL
Albumin	3.6	H	g/dL
A/G ratio	0.86		
Plasma protein	8.5	H	g/dL
Sodium	112	L	mmol/L
Potassium	3.0		mmol/L
Chloride	100		mmol/L
Total CO_2	7	L	mmol/L
Anion gap	8	L	mmol/L
	Blood Gas Analysis		
HCO_3^-	6.1	L	mEq/L
PCO_2	20.5	L	mmHg
pH	7.10	L	

www.Ebook777.com

PROBLEMS:

1. **Polycythemia**. Polycythemia (**hemoconcentration**) can result from relative or absolute increases in red cell mass. Relative polycythemia is more common and may be caused by dehydration and splenic contraction from excitement, fear, or strenuous short-term exercise (epinephrine responses). In this horse, polycythemia is the result of dehydration (the effects of which are also reflected by hyperproteinemia with a normal A/G ratio).

2. **Leukopenia, neutropenia, degenerative left shift, toxic change of neutrophils,** and **lymphopenia**. These changes in the leukogram suggest a guarded to poor prognosis and suggest an acute infection and/or endotoxemia.

a. **Leukopenia**. Neutrophils are the predominant circulating leukocyte in horses in health. Therefore, severe neutropenia may result in leukopenia.

b. **Neutropenia**. Neutropenia indicates a guarded to poor prognosis. **Neutropenia** suggests an overwhelming tissue demand for neutrophils. With severe diarrhea, the intestine may be the site of neutrophil demand. If endotoxemia is present, neutrophils may be rapidly transitioning into the marginal neutrophil pool (where they cannot be enumerated by the WBC count) and emigrating into the tissues.

c. **Degenerative left shift**. The degenerative left shift also indicates a guarded to poor prognosis. This change in the leukogram suggests that the bone marrow maturation and storage pool is depleted of segmented neutrophils and that the bone marrow apparently is unable to meet tissue demands for these phagocytes.

d. **Toxic changes of neutrophils**. The presence of toxic changes (cytoplasmic basophilia and vacuolation) suggests disturbed cellular maturation in the bone marrow. Toxic neutrophils also may have some derangements of cell function that make them less effective as phagocytes. Toxic changes are associated with marked to severe inflammation or infection.

e. **Lymphopenia.** Severe lymphopenia also contributes to the leukopenia. This change suggests marked stress with redistribution of recirculating lymphocytes. However, destruction of lymphoid tissue by infectious agents cannot be discounted.

3. **Hyperfibrinogenemia**. Extreme **hyperfibrinogenemia** also substantiates the inflammatory character of the disease. Fibrinogen is an acute phase reactant whose concentration increases in **peracute to acute inflammatory disease**. Dehydration may cause a relative increase in the fibrinogen concentration, but calculation of the **protein/fibrinogen ratio** (8.5 / 1.1 = 7.7) indicates that true hyperfibrinogenemia exists. In some individuals, hyperfibrinogenemia may precede changes in the leukogram.

4. **Azotemia**. The increased BUN concentration indicates azotemia. Dehydration is a common cause of prerenal azotemia; however, a urinalysis and other clinical information are needed to exclude renal and post-renal causes of azotemia.

5. **Hyperproteinemia, hyperalbuminemia, and normal A/G ratio**.

a. **Hyperproteinemia**. Hyperproteinemia can be relative or absolute. The former is usually caused by dehydration, whereas the latter often is associated with immunoglobulin production. Hyperfibrinogenemia is excluded because serum protein is quantified in the biochemical profile (fibrinogen is present only in plasma).

b. **Hyperalbuminemia**. The body does not produce excessive quantities of albumin. Therefore, this change is due to a relative increase in albumin concentration from dehydration.

c. N**ormal A/G ratio**. The presence of a normal A/G ratio indicates albumin and globulin concentrations have increased together as a result of dehydration.

6. **Hyponatremic dehydration**. **Hyponatremia** is particularly severe, indicating a disproportionately greater sodium loss than water loss. This laboratory finding is typical of acute diarrhea in horses. Although the serum chloride concentration is within the reference interval, total body chloride content

Free ebooks ==> www.Ebook777.com

is low (serum chloride × decreased ECF volume). Unless otherwise measured, the osmolality is presumed to be low because of the severity of the hyponatremia. A loss of K^+ with diarrhea and an internal shift of K^+ from ICF to ECF caused by acidemia depletes total body K^+, although the serum K^+ concentration is within the reference interval at this time.

7. **Metabolic acidosis**. Severe **metabolic acidosis** is present. Although respiratory compensation is indicated by the **low PCO_2**, the blood pH is dangerously low (**acidemia**). Loss of HCO_3^--rich intestinal fluid is indicated by the **normal anion gap** and normal Cl^- concentration in face of severe **hyponatremia** (relative hypochloridemia). Acidosis causing $ICF_{K+} \rightarrow ECF_{K+}$ is balanced by the loss of ECF_{K+} in diarrheal fluids at this sampling time. The normal anion gap excludes titration of HCO_3^- as a cause of the acidosis.

SUMMARY: Salmonellosis was confirmed at necropsy.

www.Ebook777.com

CASE 7 FELINE INFECTIOUS PERITONITIS

SIGNALMENT: Feline, Siamese, castrated male, 4 years old

PRESENTING PROBLEMS: Anorexia, emaciation, abdominal enlargement of 1 to 2 weeks duration, lethargy, iridal hemorrhage

LABORATORY DATA:

	Hematology		
Hct	26	L	%
Hb	8.8	L	g/dL
RBC	5.23	L	$\times 10^6/\mu L$
MCV	49.7		fL
MCH	16.8		pg
MCHC	33.8		%
Retic	0.1		%
Abs retic	52		$\times 10^3/\mu L$
RBC morphology: normal			
Platelets: adequate			
WBC	20.6	H	$\times 10^3/\mu L$
Seg	15.769 (76.5%)	H	$\times 10^3/\mu L$
Band	1.236 (6.0%)	H	$\times 10^3/\mu L$
Lymph	3.090 (15.0%)		$\times 10^3/\mu L$
Mono	0.515 (2.5%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Serum Chemistry		
Total protein	9.3	H	g/dL
Albumin	1.9	L	g/dL
α-globulin	0.5	L	g/dL
β-globulin	0.5	L	g/dL
γ-globulin	6.4	H	g/dL
A/G ratio	0.26	L	
Electrophoretic pattern: polyclonal gammopathy			
	Other Tests		
Abdominal fluid analysis: yellow-green, cloudy, viscid nucleated cell count = 5,008 cells/μl nondegenerative neutrophils, macrophages, granular proteinic background			

Free ebooks ==> www.Ebook777.com

Protein	6.0	H	g/dL
Albumin	1.5	L	g/dL
α-globulin	0.2		g/dL
β-globulin	0.3		g/dL
γ-globulin	4.0	H	g/dL
A/G ratio	0.33		

Opinion: purulent exudate consistent with feline infectious peritonitis virus infection

PROBLEMS:

1. **Anemia of chronic disorders**. A **normocytic**, **normochromic**, **nonregenerative anemia** with normal erythrocyte morphology is associated with chronic inflammation. The lack of neutropenia and thrombocytopenia exclude aplastic anemia. Erythrocytes do not have any morphologic changes, and a specific etiology of the anemia is not indicated. These findings are typical of the anemia of chronic disorders (anemia of chronic disease, anemia of chronic inflammation).

2. **Leukocytosis, neutrophilia, and left shift**. These changes indicate an **inflammatory leukogram**. **Neutrophilia** with a significant **left shift** indicates an intense but appropriate response to a tissue demand for neutrophils. A left shift is considered the hallmark of marked inflammation or infection. Peritonitis is probably responsible for the tissue demand for phagocytes.

3. **Hyperproteinemia**. The high serum protein concentration due to polyclonal increases in gamma-globulin (**polyclonal gammopathy** due to immunoglobulins) is characteristic of chronic antigenic stimulation such as that occurring with FIP viral infection. Causes of hypoalbuminemia could include loss by high-protein exudation into the peritoneal cavity, decreased production during inflammation (albumin is a negative acute phase reactant), diminished production with cachexia, or renal loss via proteinuria.

4. **Purulent peritonitis**. **Nondegenerate neutrophils** usually suggest a nonbacterial etiology; FIP virus does not produce local conditions that are conducive to degenerative changes in neutrophils. FIP causes a hypocellular, high-protein exudate. A/G ratios of <0.81 in abdominal fluid are reported to be strongly suggestive of FIP. The **fluid A/G ratio** in this case is **very low (0.33)**. The **granular background precipitate** on smears of the exudate is indicative of its high protein content.

SUMMARY: The clinical diagnosis of feline infectious peritonitis was confirmed at necropsy.

www.Ebook777.com

CASE 8 PYOMETRA

SIGNALMENT: Canine, Staffordshire Bull Terrier (pit bull terrier), female, 5 years old

HISTORY: In estrus 1 month ago

PRESENTING PROBLEMS: Anorexia, distended abdomen, vomiting, fever, PU/PD, dehydration

LABORATORY DATA:

	Hematology		
Hct	30.5	L	%
Hb	10.5	L	g/dL
RBC	4.34	L	$\times 10^6/\mu L$
MCV	70		fL
MCH	24.2		pg
MCHC	34.4		%
Retic	0.2		%
Abs retic	9		$\times 10^3/\mu L$
RBC morphology: normal			
Platelets	260		$\times 10^3/\mu L$
MPV	8.1		fL
WBC	30.8	H	$\times 10^3/\mu L$
Seg	5.852 (19%)	H	$\times 10^3/\mu L$
Band	18.172 (59%)	H	$\times 10^3/\mu L$
Metamyel	0.924 (3%)	H	$\times 10^3/\mu L$
Lymph	1.540 (5%)		$\times 10^3/\mu L$
Mono	4.312 (14%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: cytoplasmic basophilia and vacuolation			
	Serum Chemistry		
BUN	41	H	mg/dL
Creatinine	1.9	H	mg/dL
Total protein	9.2	H	g/dL
Albumin	2.3		g/dL
A/G ratio	0.33	L	
ALP	97		U/L

Free ebooks ==> www.Ebook777.com

ALT	16		U/L
Glucose	74	L	mg/dL
Sodium	128	L	mmol/L
Potassium	4.4		mmol/L
Chloride	97	L	mmol/L
Total CO_2	19		mmol/L
Anion gap	12		mmol/L
Calcium	9.7		mg/dL
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	slightly turbid		
Sp Gr	1.020		
pH	6.5		
Protein	2+		
Glucose	negative		
Ketone	negative		
Bilirubin	negative		
Blood	2+		
Sediment	20–30 RBC/hpf		
	30–40 WBC/hpf		

PROBLEMS:

1. Anemia of chronic disorders. A **normocytic**, **normochromic**, **nonregenerative anemia** with evidence of granulopoiesis and thrombopoiesis indicates a selective erythroid problem in the bone marrow. Given the evidence of chronic inflammation, the anemia of chronic disorders is most likely.

2. Leukocytosis, neutropenia, degenerative left shift, toxic changes of neutrophils, and monocytosis.

a. Leukocytosis. Leukocytosis can result from an increase in any type of leukocyte. Changes in the absolute counts of the various leukocyte subtypes identify the offending leukocyte(s) responsible for the leukocytosis. In this patient, neutrophilia is largely responsible for the leukocytosis although, monocytosis also is present.

b. Neutrophilia with a degenerative left shift. Neutrophilia with a **degenerative left shift** suggests severe inflammation **(inflammatory leukogram)** or infection. The fact that immature neutrophils outnumber the segmenters indicates that the storage pool of neutrophils is depleted and suggests a guarded prognosis.

c. Toxic changes of neutrophils. Neutrophil cytoplasmic basophilia and vacuolation are **toxic changes** that suggest bacterial infection or severe inflammation, and also call for a guarded prognosis. Toxic changes are an indication of disturbed maturation of neutrophils in the bone marrow.

d. Monocytosis may occur in either acute or chronic disease. Monocytosis may be observed with chronic inflammation, tissue necrosis, or severe stress. Severe stress (with endogenous cortisol release) is unlikely in this patient because lymphopenia is not present.

3. Azotemia. The mild azotemia (increased BUN and creatinine concentrations) may be prerenal or renal in origin; because the urine specimen is voided, postrenal azotemia is excluded. Prerenal azotemia may be due to dehydration from vomiting and insensible water loss with fever. However, the

www.Ebook777.com

decreased urine specific gravity is inappropriate because the kidneys should be attempting to conserve body water by concentrating the urine. Renal function should be evaluated more critically.

4. **Hyperproteinemia, normoalbuminemia, and decreased A/G ratio**.
   a. **Hyperproteinemia** may be relative (e.g., dehydration) or absolute (e.g., increased globulin production).
   b. Because **normoalbuminemia** is present, the hyperproteinemia may be due to hyperglobulinemia. Alternatively, proteinuria could indicate renal loss of albumin and hypoalbuminemia may be masked by concurrent dehydration.
   c. A **decreased A/G ratio** in serum can occur from selective albumin loss, increased globulin production, or both. In this patient, **hyperglobulinemia** is probably due to antigenic stimulation associated with pyometra. Serum protein electrophoresis probably would reveal a broad-based or polyclonal gammopathy, perhaps with increased concentration of acute-phase proteins (α-and β-globulins). Dehydration may have contributed to the hyperproteinemia but would not have altered the A/G ratio. Proteinuria, especially of glomerular origin, may cause selective albumin loss, which also would tend to decrease the A/G ratio.

5. **Hypoglycemia**. Increased tissue utilization of glucose can occur with sepsis.

6. **Hyponatremic dehydration** and **hypochloridemia**. Excessive diuresis and vomiting are responsible for these laboratory abnormalities. Osmolality is estimated to be 283 mOsm/kg $[2(Na^+ + K^+) + (BUN \div 2.8) + (glucose \div 18)]$. Kaliuresis and loss of K^+ in vomitus are expected, but an internal shift of K^+ ions is not likely at this time.

7. **Low urine specific gravity, pyuria, hematuria, proteinuria**.
   a. The **low urine Sp Gr** in the presence of dehydration indicates the inability of the kidneys to concentrate urine and conserve body water. This observation may be the result of endotoxin production in pyometra. The endotoxin prevents the collecting tubular epithelial cells from responding to ADH to conserve water, resulting in polyuria. However, primary renal disease cannot be excluded.
   b. A **voided urine specimen** may be **contaminated** by uterine exudate, resulting in **potential pyuria, hematuria**, and **proteinuria**. A urine specimen obtained by catheterization or cystocentesis may confirm or refute urinary tract inflammation or infection as a cause of these abnormalities.
   c. **Proteinuria** could be caused by contamination of the urine specimen with genital exudates, urinary tract inflammation, or a renal lesion involving the glomerulus or tubules. Further diagnostic testing may be necessary to identify the source of the proteinuria.

SUMMARY: This patient had pyometra. Ovariohysterectomy removed the cause and source of inflammation and infection. The left shift diminished initially, but the neutrophilia intensified for 1 to 2 days before returning to the reference interval over the next several days. Following surgical extirpation of the site of tissue demand for neutrophils, the blood neutrophil count will escalate until granulopoiesis is down-regulated.

Free ebooks ==> www.Ebook777.com

CASE 9 FELINE INFECTIOUS PANLEUKOPENIA (PARVOVIRUS INFECTION)

SIGNALMENT: Feline, domestic shorthair, male, 10 months old

PRESENTING PROBLEMS: Vomiting and profuse diarrhea for 18 hours, dehydration estimated at 7%, pyrexia, anorexia, gingivitis, enlarged peripheral lymph nodes

LABORATORY DATA:

	Hematology		
Hct	49	H	%
Hb	15.8	H	g/dL
RBC morphology: normal			
Platelets: adequate			
WBC	1.9	L	$\times 10^3/\mu L$
Seg	0.038 (2%)	L	$\times 10^3/\mu L$
Band	0.057 (3%)		$\times 10^3/\mu L$
Lymph	0.513 (24%)	L	$\times 10^3/\mu L$
Mono	1.349 (71%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	cystocentesis		
Color	yellow		
Turbidity	clear		
Sp Gr	1.065		
pH	6.0		
Chemistries	normal		
Sediment	none		
	Serum Chemistry		
BUN	46	H	mg/dL
Total protein	8.0	H	g/dL
Albumin	3.4	H	g/dL
A/G ratio	0.74		

continued

www.Ebook777.com

Sodium	148		mmol/L
Potassium	4.2		mmol/L
Chloride	126		mmol/L
Total CO_2	10	L	mmol/L
Anion gap	12.2		mmol/L

Other Tests

FeLV	negative

Bone marrow aspirate: hypocellular particles, low M/E ratio, normal erythroid maturation, only early stages in myeloid series, adequate megakaryocytes.
Opinion: granulocytic hypoplasia, early recovery from parvovirus infection
Lymph node aspirate: mostly small lymphocytes, many plasma cells, some large lymphocytes.

Opinion: lymphoid hyperplasia

PROBLEMS:

1. **Leukopenia, neutropenia** with a **degenerative left shift**, and **granulocytic hypoplasia of the bone marrow**.

a. **Leukopenia** in monogastric animals may be caused by severe neutropenia.

b. **Neutropenia** can be caused by decreased production of neutrophils, overwhelming tissue consumption of neutrophils, or a transient shift of neutrophils from the circulating neutrophil pool (where they are enumerated by the WBC count) to the marginal neutrophil pool (where they cannot be counted). Neutropenia is probably multifactorial, including decreased neutrophil production from viral destruction of hematopoietic cells, an intense tissue demand for neutrophils because of viral enteritis, and endotoxemia from enteritis with a shift of neutrophils from the circulating neutrophil pool to the marginal neutrophil pool.

c. The **degenerative left shift** indicates that the bone marrow maturation and storage pool of neutrophils is depleted of segmenters and that the bone marrow granulopoiesis is failing to meet tissue demands for neutrophils.

d. The **low M/E ratio** in the bone marrow aspirate, in conjunction with **leukopenia**, **neutropenia**, and hematocrit within the reference interval, indicates **granulocytic hypoplasia** with decreased production of neutrophils.

2. **Lymphopenia**. Lymphopenia is a common finding in acute systemic infections, especially viral disease. Parvovirus infects and destroys rapidly dividing cell populations including lymphocytes, hematopoietic cells, and intestinal crypt epithelial cells. Lymphopenia also may be due to stress and transient redistrubution of lymphocytes. Recirculating lymphocytes may be entrapped within lymph nodes, promoting antigen exposure and amplifying the immune response.

3. **Monocytosis**. Monocytosis can occur in acute or chronic disease. Monocytosis also may herald the resolution of neutropenia because monocytes are released from the bone marrow into the blood at a relatively young stage of development. In contrast, neutrophils will mature for a variable time period before being released from the bone marrow.

4. **Prerenal azotemia**. An **increased BUN concentration** with **dehydration** and **very concentrated urine** suggests decreased renal perfusion with a decreased glomerular filtration rate. When dehydration is present, the kidneys are maximally stimulated to conserve body water.

5. **Hyperproteinemia, hyperalbuminemia, hyperglobulinemia, and normal A/G ratio**.

a. **Hyperproteinemia** is probably a relative increase in protein concentration because of plasma water loss from dehydration.

Free ebooks ==> www.Ebook777.com

b. **Hyperalbuminemia** occurs with dehydration; the body does not overproduce albumin.
c. The **normal A/G ratio** indicates that both globulin and albumin concentrations are increased in the same proportion (**panhyperproteinemia**). This change occurs only with dehydration.

6. **Normonatremic dehydration, normokalemia, and acidosis**.
a. The degree of **dehydration**, determined on clinical examination, is severe enough to have caused **polycythemia** (hemoconcentration), **hyperproteinemia**, **azotemia**, and **very concentrated urine**. Na^+ has been lost in proportion to body water, leading to isotonic dehydration.
b. Despite **normokalemia**, a negative potassium balance probably exists. Negative external K^+ balance is due to losses of body fluids. In addition, an internal shift of K^+ from ICF to ECF is suspected because of severe acidosis. An estimate of total body K^+ cannot be made from these data, but K^+ deficit is likely in cases of vomiting, diarrhea, and acidemia.
c. **Low HCO_3^-** with a normal anion gap and Cl^- concentration denotes loss of bicarbonate-(HCO_3^-) rich intestinal fluid.

7. **Lymph node hyperplasia**. Fine-needle aspiration can determine the relative distribution of cell types within the lymph node. In this patient, fine-needle aspiration revealed lymphoid hyperplasia that probably occurred secondary to infection or some other form of antigenic stimulation. Lymphoid hyperplasia rarely is associated with lymphocytosis in the blood. Lymph node enlargement also can occur with edema and concurrent lymphocellular depletion.

SUMMARY: The diagnosis of parvovirus infection (feline infectious panleukopenia) was based on clinical signs and laboratory findings. This virus causes destruction of all hematopoietic precursors approximately 3 to 4 days after viral infection. Neutropenia usually is present when clinical signs of disease appear. Because of the short circulating half life of neutrophils and an increased demand for these cells in the presence of enteritis, neutropenia occurs more consistently than anemia or thrombocytopenia. Anemia, if present, may be masked by concurrent dehydration from vomiting and/or diarrhea. Persistent neutropenia may be a consequence of viral enteritis, endotoxemia, and/or destruction of granulopoietic precursor cells.

www.Ebook777.com

CASE 10 MULTICENTRIC LYMPHOMA

SIGNALMENT: Canine, Saint Bernard, male, 4 years old

PRESENTING PROBLEMS: Bilateral enlargement of submandibular, prescapular, and popliteal lymph nodes

LABORATORY DATA:

	Hematology		
Hct	31	L	%
Hb	10.0	L	g/dL
RBC	4.22	L	$\times 10^6/\mu L$
MCV	73.4		fL
MCH	23.6		pg
MCHC	32.2		%
Retic	0		%
Abs retic	0		$\times 10^3/\mu L$
RBC morphology: normal			
Platelets: adequate			
WBC	23.4	H	$\times 10^3/\mu L$
Seg	19.539 (83.5%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	1.053 (4.5%)		$\times 10^3/\mu L$
Mono	2.691 (11.5%)	H	$\times 10^3/\mu L$
Eos	0.117 (0.5%)		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: lymphoblasts			
	Other tests		
Bone marrow aspirate: normocellular particles adequate megakaryocytes slightly increased M/E ratio normal erythroid and myeloid maturation approximately 7% small lymphocytes			
Opinion: granulocytic hyperplasia equivocal erythroid hypoplasia absence of neoplastic involvement			
Lymph node aspirate: monomorphic population of lymphoblasts			
Opinion: lymphoma			
	Serum Chemistry		
BUN	61	H	mg/dL
Creatinine	6.9	H	mg/dL
Total protein	6.9		g/dL

Free ebooks ==> www.Ebook777.com

Albumin	3.2		g/dL
A/G ratio	0.87		
ALP	84		U/L
ALT	46		U/L
Glucose	98		mg/dL
Sodium	153		mmol/L
Potassium	4.5		mmol/L
Chloride	117		mmol/L
Total CO_2	22		mmol/L
Anion gap	18.2		mmol/L
Calcium	19.1	H	mg/dL
Phosphorus	4.5		mg/dL
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	cloudy		
Sp Gr	1.011		
pH	6.0		
Protein	trace		
Other chemistries	negative		
Sediment	1 to 2 granular casts/hpf 0 to 2 WBC/hpf		

PROBLEMS:

1. **Lymphoma with leukemic blood profile**. The diagnosis of lymphoma is evident from clinical signs and the presence of **lymphoblasts** in the blood and in lymph node aspirates. Neoplastic lymphocytes are not evident in bone marrow aspirates. Peripheral lymphadenopathy and a leukemic profile indicate dissemination of lymphoma.

2. **Anemia of chronic disorders**. **Normocytic, normochromic, nonregenerative anemia** with an absence of neutropenia and thrombocytopenia suggests **erythroid hypoplasia**, which was confirmed by bone marrow examination. Anemia is probably secondary to lymphoma.

3. **Hypercalcemia**. Hypercalcemia (hypercalcemia of malignancy) occurs in approximately 10% to 40% of dogs with lymphoma and is associated with bone resorption.

4. **Renal failure**. Renal failure is identified by **azotemia** that is associated with **isosthenuria**. The presence of **granular casts** indicates a renal tubular lesion of unknown severity. Renal failure in this dog is probably the result of nephrocalcinosis from prolonged hypercalcemia.

SUMMARY: Multicentric lymphoma is the primary disease; the other problems are secondary conditions or paraneoplastic syndromes.

www.Ebook777.com

CASE 11 RENAL ABSCESS AND SECONDARY DISSEMINATED INTRAVASCULAR COAGULATION

SIGNALMENT: Canine, English Bulldog, spayed female, 2 years old

HISTORY: Dexamethasone administered within the past 24 hours

PRESENTING PROBLEMS: Epistaxis, oral ecchymotic hemorrhages, palpable abdominal mass, abdominal pain

LABORATORY DATA:

	Hematology		
Hct	25	L	%
Hb	8.3	L	g/dL
RBC	3.88	L	$\times 10^6/\mu L$
MCV	64		fL
MCH	21		pg
MCHC	33		%
Retic	0.8		%
Abs retic	31		$\times 10^3/\mu L$
RBC morphology: schistocytes			
Platelets	83	L	$\times 10^3/\mu L$
MPV	12	H	fL
WBC	35.3	H	$\times 10^3/\mu L$
Seg	23.827 (67.5%)	H	$\times 10^3/\mu L$
Band	7.060 (20.0%)	H	$\times 10^3/\mu L$
Metamyel	0.529 (1.5%)	H	$\times 10^3/\mu L$
Lymph	0.709 (2.0%)		$\times 10^3/\mu L$
Mono	3.001 (8.5%)	H	$\times 10^3/\mu L$
Eos	0.179 (0.5%)		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			

Free ebooks ==> www.Ebook777.com

	Serum Chemistry		
BUN	10		mg/dL
Creatinine	0.8		mg/dL
Total protein	8.3	H	g/dL
Albumin	2.6		g/dL
A/G ratio	0.46	L	
ALP	96		U/L
ALT	62		U/L
Glucose	83		mg/dL
	Other Tests		
Modified Knott's test:negative			
BMBT	11.0	H	min
APTT	42.2	H	sec
PT	19.1	H	sec
TT	18.2	H	sec
FDP	>40	H	µg/mL

PROBLEMS:

1. **Anemia of chronic disorders**. **Normocytic, normochromic, nonregenerative anemia** associated with adequate numbers of neutrophils suggests a selective erythrocytic disorder affecting the bone marrow. The anemia of chronic disease is most likely because of selective erythroid depression in the face of an inflammatory leukogram and hyperglobulinemia. Serum iron levels would be expected to be low and bone marrow macrophage iron increased. Although the anemia is nonregenerative, the presence of **schistocytes** suggests a hemolytic component due to fragmentation of erythrocytes.

2. **Inflammatory leukogram**.
   a. **Neutrophilia** with prominent **left shift** (metamyelocytes in circulation) suggests inflammation and excessive demand for neutrophils.
   b. **Monocytosis** is consistent with inflammation or may be a response to corticosteroids. Monocytosis occurs frequently in dogs after corticosteroid administration.
   c. The **lymphocyte count** appears slightly decreased but is still **within the reference interval**. The lymphocyte count usually returns to the reference interval within 24 hours after administration of a single dose of a short-acting corticosteroid.

3. **Disseminated intravascular coagulation (DIC)**.
   a. **Thrombocytopenia** can occur from lack of platelet production, increased platelet consumption, or immune-mediated platelet destruction. Consumptive thrombocytopenia is observed in uncompensated DIC. If thrombocytopenia was the only hemostatic abnormality in this dog, it would probably be insufficient to cause hemorrhage.
   b. The **increased mean platelet volume (MPV)** indicates increased platelet turnover. Younger platelets generally are larger than older platelets.
   c. **Prolonged buccal mucosal bleeding time (BMBT)** may be a consequence of thrombocytopenia, but also may reflect platelet dysfunction caused by FDPs coating platelet surfaces.
   d. In uncompensated DIC, **consumption** of the nonenzymatic clotting factors occurs. These include **factor V**, **factor VIII**, and **fibrinogen (factor I)**. Consumption of these factors will result in a **prolonged activated partial thromboplastin time (APTT)**, **prothrombin time (PT)**, and **thrombin time (TT)**.

www.Ebook777.com

e. **Hypofibrinogenemia** is indicated by prolongation of the APTT, PT, and TT, above. A prolonged TT is the most sensitive measure of decreased fibrinogen concentration. The heat precipitation technique will detect hyperfibrinogenemia, but is too insensitive to accurately detect hypofibrinogenemia.

f. **Increased concentration of fibrin(ogen) degradation products (FDPs)** indicates excessive fibrin clot lysis and degradation of fibrinogen. Excessive FDPs may interfere with platelet function (prolonged BMBT) and the TT test.

g. **Fibrin strands** that are formed in the microvasculature will shred and otherwise damage erythrocytes, resulting in the formation of **schistocytes.**

4. **Hyperglobulinemia**. Prolonged antigenic stimulation associated with inflammation can cause a polyclonal increase in immunoglobulins. **Hyperproteinemia** with a **low A/G ratio** indicates hyperglobulinemia; electrophoresis is necessary to confirm the polyclonal (broad-based globulin spike) nature of the increase.

SUMMARY: A unilateral renal abscess was the cause of the inflammation and subsequent uncompensated DIC. A lesion affecting only one kidney is insufficient to cause signs of renal failure (e.g., azotemia).

Free ebooks ==> www.Ebook777.com

CASE 12 HEPATIC ENCEPHALOPATHY (HEPATIC FIBROSIS)

SIGNALMENT: Equine, Tennessee Walking Horse, mare (female), 20 years old

PRESENTING PROBLEMS: Anorexia, circling, ataxia, yawning, head pressing (pushing against objects), muscle fasciculation, icterus

LABORATORY DATA:

	Hematology		
Hct	35.4		%
Hb	13.6		g/dL
RBC	7.64		$\times 10^6/\mu L$
MCV	46		fL
MCH	17.8		pg
MCHC	38.4		%
RBC morphology: normal			
Platelets: adequate			
WBC	20.6	H	$\times 10^3/\mu L$
Seg	16.686 (81%)	H	$\times 10^3/\mu L$
Band	1.854 (9%)	H	$\times 10^3/\mu L$
Lymph	1.648 (8%)		$\times 10^3/\mu L$
Mono	0.206 (1%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.206 (1%)		$\times 10^3/\mu L$
WBC morphology: normal			
Fibrinogen	800	H	mg/dL
	Serum Chemistry		
BUN	2	L	mg/dL
Creatinine	0.6		mg/dL
Total protein	7.0		g/dL
Albumin	2.9		g/dL

continued

www.Ebook777.com

A/G ratio	0.70		
GGT	251	H	U/L
AST	580	H	U/L
SDH	54	H	U/L
Glucose	110		mg/dL
Sodium	132		mmol/L
Potassium	3.2		mmol/L
Chloride	101		mmol/L
Total CO_2	24		mmol/L
Anion gap	10.2	H	mmol/L
Calcium	10.8		mg/dL
T. bilirubin	28.2	H	mg/dL
D. bilirubin	5.6	H	mg/dL
Bile acids	202	H	µmol/L
NH_3	79	H	µg/dL

PROBLEMS:

1. **Hepatic disease**.
   a. **Increased SDH** and **AST** activities indicate **hepatocellular** disease with **leakage** of cytosolic enzymes into the plasma. Although AST activity is increased in muscle disease, the association with increased SDH activity suggests hepatic origin. Muscle involvement could be refuted or confirmed by CK analysis.
   b. **Hyperbilirubinemia** and **increased GGT activity** indicate cholestasis. **Unconjugated (indirect) bilirubin** predominates in all cases of hyperbilirubinemia in the horse. In the absence of anemia, hyperbilirubinemia suggests hepatic disease. Anorexia also can cause hyperbilirubinemia in the horse, but hyperbilirubinemia of this magnitude is not caused by anorexia alone.
   c. The **increased bile acids** suggest cholestasis or decreased hepatic functional mass.
   d. **Hyperammonemia** and **decreased BUN** concentration also suggest decreased hepatic functional mass with failure to convert ammonia to urea via the hepatic urea cycle. However, the decrease in hepatic functional mass has not affected albumin synthesis.

2. **Inflammatory leukogram**. **Neutrophilia** with a **left shift** suggests inflammation and an intense tissue demand for neutrophils.

3. **Hyperfibrinogenemia** is a marker of inflammation, especially in large animals. Inflammation may provoke hyperfibrinogenemia before changes in the leukogram are apparent.

SUMMARY: Necropsy and histopathology disclosed discrete hepatocellular necrosis with periportal to bridging fibrosis and biliary hyperplasia. In horses, plant toxicosis may cause these hepatic lesions. Purulent colitis also was present.

Free ebooks ==> www.Ebook777.com

CASE 13 PORTOSYSTEMIC VENOUS SHUNT WITH HEPATIC ATROPHY

SIGNALMENT: Canine, Lhasa Apso, spayed female, 5 years old

PRESENTING PROBLEMS: Depression, lethargy, seizures, ataxia, anorexia, PU/PD

RADIOGRAPHIC FINDINGS: Decreased hepatic shadow

LABORATORY DATA:

	Hematology		
Hct	39.1		%
Hb	13.7		g/dL
RBC	6.90		$\times 10^3/\mu L$
MCV	57	L	fL
MCH	19.9		pg
MCHC	35.0		%
Retic	ND		
RBC morphology: codocytes (target cells)			
Platelets	237		$\times 10^3/\mu L$
WBC	9.8		$\times 10^3/\mu L$
Seg	7.548 (77%)		$\times 10^3/\mu L$
Band	0.588 (5%)		$\times 10^3/\mu L$
Lymph	1.176 (12%)		$\times 10^3/\mu L$
Mono	0.098 (1%)		$\times 10^3/\mu L$
Eos	0.392 (4%)		$\times 10^3/\mu L$
Baso	0.098 (1%)		$\times 10^3/\mu L$
WBC morphology: normal			
	Serum Chemistry		
BUN	2	L	mg/dL
Creatinine	0.4		mg/dL
Total protein	3.7	L	g/dL
Albumin	1.1	L	g/dL
A/G ratio	0.43	L	
ALP	181	H	U/L
ALT	122	H	U/L
Glucose	52	L	mg/dL
Sodium	145		mmol/L

continued

www.Ebook777.com

Potassium	4.1		mmol/L
Chloride	118		mmol/L
Total CO_2	21		mmol/L
Anion gap	5		mmol/L
Calcium	8.2	L	mg/dL
Phosphorus	4.1		mg/dL
T. bilirubin	0.9	H	mg/dL
Bile acids (fast)	18.6	H	µmol/L
Bile acids (post)	246.1	H	µmol/L
NH_3 (fast)	438	H	µg/dL
Cholesterol	45	L	mg/dL
	Urinalysis		
Urine source	cystocentesis		
Color	straw		
Turbidity	clear		
Sp Gr	1.006		
pH	8.0		
Protein	1+		
Glucose	negative		
Ketone	negative		
Bilirubin	3+		
Blood	negative		
Sediment	ammonium biurate crystals		

PROBLEMS:

1. **Hepatic failure**.
   a. **Increased bile acid concentrations** (**fasting and postprandial**). Increased bile acid concentrations suggest hepatic failure and/or portosystemic shunting of blood. In health, 95% of bile acids are removed from portal blood and recycled by the liver.
   b. **Fasting hyperammonemia** and **decreased BUN** concentration. Both liver failure and portosystemic shunts result in decreased ammonia uptake and conversion to urea by hepatocytes. Hyperammonemia may cause CNS signs (hepatic encephalopathy) including depression, ataxia, and seizures. The toxicity of ammonia (NH_3) is increased in alkalosis, and decreased in acidosis (NH_4^+ is relatively nondiffusable and nontoxic).
   c. **Hypoproteinemia, hypoalbuminemia,** and decreased **A/G ratio**. These findings indicate decreased hepatic synthesis of albumin. However, 1+ proteinuria in dilute urine may represent significant renal loss of albumin in this patient.
   d. **Hypoglycemia.** Fasting hypoglycemia suggests hyperinsulinism (e.g., β-cell tumor of the pancreas) or decreased hepatic glycogen stores needed to maintain glucose homeostasis. Other biochemical abnormalities suggest that the hypoglycemia is due to hepatic dysfunction. Because the liver is the primary depot of glycogen storage, animals with hepatic insufficiency often have prolonged post-prandial hyperglycemia followed by hypoglycemia during periods of fasting.
   e. **Hypocholesterolemia**. The liver is the primary site of cholesterol production. Reduced hepatic synthesis of cholesterol may occur with decreased hepatic functional mass.

2. **Increased ALT activity**. Mildly elevated ALT activity indicates hepatocellular injury with enzyme leakage from the cytosol of hepatocytes into the blood. The magnitude of ALT activity does not indicate the severity or duration of the hepatic lesion.

Free ebooks ==> www.Ebook777.com

3. **Hyperbilirubinemia**. Hyperbilirubinemia indicates hepatobiliary disease. Hemolysis probably can be excluded as the cause of the hyperbilirubinemia because the hematocrit is within the reference interval and reticulocytosis is not observed.

4. **Mildly increased ALP activity**. Increased ALP activity probably indicates **cholestasis**. The activity of this enzyme may be induced by stasis of bile flow. In dogs, ALP activity also may increase following endogenous cortisol release or corticosteroid treatment.

5. **Hypocalcemia**. The decreased calcium concentration is probably due to hypoalbuminemia with a reduction in protein-bound calcium. The correction formula for hypoproteinemia places the calcium value within the reference interval: (8.2 - 0.4[3.7] + 3.3 = 10.02). Determination of the ionized calcium concentration would provide more accurate information on calcium status.

6. **Microcytosis**. This finding is common in portosystemic venous shunts, but the cause is unknown. Microcytosis may be observed in health in some Asian breeds of dogs including Akita, Chow Chow, Shar Pei, and Shiba Inu.

7. **Hyposthenuria**. Dilute urine is probably due to lack of medullary tonicity from the decreased BUN concentration and medullary washout from polyuria.

8. **Ammonium biurate crystalluria**. Hyperammonemia may lead to precipitation of ammonium biurate crystals in alkaline urine.

9. **Bilirubinuria**. Urine reagent strips (dipsticks) are very sensitive in detecting conjugated bilirubin in the urine. Bilirubinuria suggests cholestasis because laboratory evidence of hemolysis and hepatic insufficiency is not present. Bilirubinuria usually is detected before hyperbilirubinemia in cholestasis because of the low renal threshold for conjugated bilirubin in dogs.

10. **Proteinuria**. A 1+ proteinuria in dilute urine is clinically significant. Proteinuria in the absence of hematuria or pyuria suggests renal protein loss from either a glomerular or tubular lesion. Reagent strip protein pads may give a false positive reaction in alkaline urine; therefore, the suspected proteinuria should be verified by another test such as acid precipitation (Robert's reagent test). Determination of the urine protein/urine creatinine ratio may further substantiate renal proteinuria and may indicate whether the protein loss is glomerular or tubular in origin.

SUMMARY: An extrahepatic portovenous shunt was observed during exploratory laparotomy. A liver biopsy revealed hepatic cord atrophy with microvascular dysplasia. These histologic changes are typical of livers from animals with portovenous shunts.

www.Ebook777.com

CASE 14 ACUTE PANCREATIC NECROSIS

SIGNALMENT: Canine, Labrador Retriever, castrated male, 10 years old

PRESENTING PROBLEMS: Obese, distended abdomen, abdominal pain, depression, vomiting, PU/PD, dehydration

LABORATORY DATA:

	Hematology		
Hct	51.4		%
Hb	16.1		g/dL
RBC	7.21		$\times 10^6/\mu L$
MCV	71.3		fL
MCH	22.3		pg
MCHC	31.3		%
RBC morphology: normal			
Platelets: adequate			
WBC	24.6	H	$\times 10^3/\mu L$
Seg	20.910 (85%)	H	$\times 10^3/\mu L$
Band	0.738 (3%)	H	$\times 10^3/\mu L$
Lymph	1.230 (5%)		$\times 10^3/\mu L$
Mono	1.722 (7%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: cytoplasmic basophilia and vacuolation			
	Serum Chemistry		
BUN	88	H	mg/dL
Creatinine	3.8	H	mg/dL
Total protein	8.9	H	g/dL
Albumin	4.0	H	g/dL
A/G ratio	0.82		
ALP	616	H	U/L
ALT	112	H	U/L
Glucose	185	H	mg/dL

Free ebooks ==> www.Ebook777.com

Sodium	148		mmol/L
Potassium	4.2		mmol/L
Chloride	115		mmol/L
Calcium	8.3	L	mg/dL
Total CO_2	21		mmol/L
Anion gap	16.2		mmol/L
Amylase	6021	H	U/L
Lipase	1400	H	U/L
	Urinalysis		
Urine source	cystocentesis		
Color	yellow		
Turbidity	clear		
Sp Gr	1.045		
pH	7.0		
Protein	negative		
Glucose	1+		
Ketone	negative		
Bilirubin	1+		
Blood	negative		
Sediment	amorphous crystals		

PROBLEMS:

1. **Inflammatory leukogram**.
   a. The **neutrophilia** and **mild left shift** reflect a tissue demand for phagocytes. Purulent inflammation is occurring in response to pancreatic inflammation and necrosis, peritonitis, and mesenteric steatitis caused by the spillage of pancreatic enzymes.
   b. The **toxic changes** in neutrophils indicates systemic toxemia caused by the pancreatitis with subsequent disturbances of neutrophil maturation within the bone marrow.
   c. **Monocytosis** may be a response to tissue necrosis and a demand for phagocytes.

2. **Prerenal azotemia**. **Azotemia** associated with an **increased urine Sp Gr** and the presence of clinical dehydration suggests decreased renal perfusion and decreased glomerular filtration of urea.

3. **Pancreatic necrosis**. **Hyperamylasemia** (6-fold increase in activity) and **hyperlipasemia** (4-fold increase in activity) of this magnitude suggest a pancreatic origin of the increased serum enzymatic activity. The decreased glomerular filtration rate, as suggested by the prerenal azotemia, may be partially responsible for the increased activity of these enzymes because of delayed renal inactivation and excretion.

4. **Cholestasis** and **hepatocellular injury**. In dogs, the common bile duct of the liver and the main pancreatic duct enter the duodenum in close proximity. Pancreatitis can be associated with inflammation and partial obstruction of the common bile duct.
   a. **Increased ALP activity** is induced by cholestasis.
   b. **Increased ALT activity** indicates hepatocellular injury. Because retained bile in the canaliculi exerts a detergent effect on cell membranes, hepatoceulluar cytosolic enzymes will leak into the sinusoidal blood. In addition, enzyme release from the pancreas into the peritoneal cavity also can damage the liver and other viscera on contact.

5. **Mild bilirubinuria** also reflects cholestasis. Bilirubinuria is detected before hyperbilirubinemia is apparent.

www.Ebook777.com

6. **Hyperproteinemia**, **hyperalbuminemia**, and **normal A/G ratio.** Dehydration causes a concomitant, relative increase in the concentration of albumin and globulins; the A/G ratio remains within the reference interval. The hematocrit percentage, hemoglobin concentration, and red blood cell count are probably increased slightly but remain within reference intervals.

7. **Hyperglycemia with mild glucosuria**. Hyperglucagonemia, which is associated with pancreatic necrosis, is the most likely cause of the hyperglycemia. Other contributing factors could include a failure of the residual pancreas to synthesize adequate quantities of insulin, and catecholamine release from the adrenal gland. The renal threshold for glucose in the dog is 180 mg/dl; therefore, this threshold has been exceeded, leading to glucosuria. Diabetes mellitus could be excluded definitively by measuring serum fructosamine concentration and/or glycated hemoglobin concentration.

8. **Hypocalcemia**. Glucagon released from the necrotic pancreas can stimulate thyrocalcitonin Secretion, which reduces blood calcium concentration. The precipitation of calcium during saponification of fat may have a minor role in the development of hypocalcemia.

SUMMARY: Extensive pancreatic necrosis was observed at necropsy.

Free ebooks ==> www.Ebook777.com

CASE 15 EXOCRINE PANCREATIC INSUFFICIENCY, DIABETES MELLITUS, AND CHRONIC RENAL FAILURE

SIGNALMENT: Canine, Poodle, female, 5 years old

PRESENTING PROBLEMS: Vomiting, weight loss, PU/PD, dehydration, acholic feces (gray-colored feces that lack bile pigments)

LABORATORY DATA:

	Hematology		
Hct	29.0	L	%
Hb	9.6	L	g/dL
RBC	4.12	L	$\times 10^6/\mu L$
MCV	70.4		fL
MCH	23.3		pg
MCHC	33.1		%
Retic	0		%
Abs retic	0		$\times 10^3/\mu L$
RBC morphology: normal			
Platelets: adequate			
WBC	13.8		$\times 10^3/\mu L$
Seg	12.144 (86%)		$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	1.242 (9%)		$\times 10^3/\mu L$
Mono	0.690 (5%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	slightly cloudy		
Sp Gr	1.012		
pH	5.5		

continued

www.Ebook777.com

Protein	negative		
Glucose	4+		
Ketone	moderate		
Bilirubin	1+		
Blood	negative		
Sediment	2 to 4 RBC/hpf 2 to 3 WBC/hpf transitional cells		
	Serum Chemistry		
BUN	135	H	mg/dL
Creatinine	3.0	H	mg/dL
Total protein	7.3		g/dL
Albumin	3.3		g/dL
A/G ratio	0.83		
ALP	990	H	U/L
ALT	118	H	U/L
Glucose	740	H	mg/dL
Sodium	136	L	mmol/L
Potassium	3.6		mmol/L
Chloride	91	L	mmol/L
Total CO_2	14	L	mmol/L
Anion gap	34.6	H	mmol/L
Calcium	9.0	L	mg/dL
Phosphorus	9.2	H	mg/dL
Total bilirubin	0.3		mg/dL
	Other Tests		
Feces: Trypsin Fat (direct) 3+	absent		
TLI	1.6	L	µg/L
Folate	17.5	H	µg/L
Cobalamin	180	L	ng/L
BT-PABA (peak conc.)	0.8	L	µmol/L

PROBLEMS:

1. **Exocrine pancreatic insufficiency**.
   - **a. Fecal screening tests** for the presence of fat and trypsin activity suggest a **deficiency** in **lipase** and **trypsin** (protease), respectively. Fecal trypsin digestion test results may be negative with pancreatic exocrine insufficiency, during inactivity of a healthy pancreas (trypsin production may be intermittent), or when trypsin is inactivated in the intestine or in the feces by bacterial proteases prior to laboratory testing. The fecal trypsin digestion test is of limited usefulness since improved diagnostic tests have been introduced.
   - **b. Decreased serum TLI and BT-PABA cleavage** (<26.2 µmol/L) indicate a deficiency in trypsinogen and chymotrypsin, respectively.
   - **c. High folate and low cobalamin concentrations** could reflect intestinal bacterial overgrowth secondary to a lack of the bacteriostatic effect of pancreatic enzymes.

Free ebooks ==> www.Ebook777.com

2. **Diabetes mellitus**. **Hyperglycemia**, **glucosuria**, and **ketonuria** are laboratory indicators of insulin deficiency. Ketonuria is detected more easily than ketonemia and indicates a negative energy balance.

3. **Hepatocellular disease**.
   a. **Increased ALP activity** suggests intrahepatic cholestasis due to compression of bile canaliculi by swollen hepatocytes; however, some dogs with diabetes also have increased ALP activity due to the induction of the steroid isoenzyme. In diabetes mellitus, a negative energy balance can promote both hepatocellular glycogen loading and hepatic lipidosis as glucose is produced from amino acids (gluconeogenesis) and lipids are consumed for energy (β-oxidation of lipids).
   b. **Increased ALT activity** indicates increased hepatocellular membrane permeability.
   c. **Bilirubinuria** reflects the cholestasis and precedes the development of hyperbilirubinemia.

4. **Renal failure**.
   a. **Isosthenuria** is characterized by a urine **Sp Gr** that ranges from **1.008 to 1.012**. The kidney is neither diluting (Sp Gr < 1.008) nor concentrating (Sp Gr > 1.012) the urine. The presence of clinical dehydration and fixed urine Sp Gr indicate **renal failure**. During dehydration, the kidney should be stimulated to conserve body water by concentrating the urine.
   b. **Azotemia** results from a decreased glomerular filtration rate (GFR). Azotemia may occur from prerenal, renal, or post-renal origin. Post-renal azotemia is excluded because the urine specimen is voided. Prerenal azotemia is possible with dehydration; however, lack of concentration of the urine also indicates renal azotemia.
   c. **Hyperphosphatemia** and **hypocalcemia** are associated with renal disease. These changes may be due to decreased production of 1,25-dihydroxycholecalciferol by the diseased kidney. The **Ca × P product is high** ($9.0 \times 9.2 = 82.8$), suggesting a propensity for soft tissue mineralization that may include nephrocalcinosis.

5. **Anemia of chronic renal disease**. **Normocytic**, **normochromic**, **nonregenerative anemia** suggests a deficiency of erythropoietin due to chronic renal disease.

6. **Metabolic acidosis with an increased anion gap**. A **decreased TCO_2** concentration and **high anion gap** indicate a titration acidosis. In this case uremic acids and ketoacids are titrating plasma bicarbonate. The increased concentration of unmeasured anions from uremia and ketoacidosis have elevated the anion gap.

7. **Hyponatremic dehydration, normokalemia, and hypochloridemia**. Na^+ and Cl^- are lost by osmotic diuresis caused by glucosuria and renal disease. K^+ loss is occurring, but the internal shift of $ICF_{K+} \rightarrow ECF_{K+}$, induced by acidosis, is maintaining normokalemia. Chloride also may be lost via vomiting.

SUMMARY: The dog responded to oral supplementation with pancreatic enzymes and insulin.

www.Ebook777.com

CASE 16 INFLAMMATORY BOWEL DISEASE (MALABSORPTION WITH PROTEIN-LOSING ENTEROPATHY)

SIGNALMENT: Canine, German Shepherd, spayed female, 5 years old

PRESENTING PROBLEMS: Diarrhea of 2 months duration, weight loss, good appetite

LABORATORY DATA:

	Hematology		
Hct	39.0		%
RBC morphology: normal			
Platelets: adequate			
WBC	11.34		$\times 10^3/\mu L$
Seg	9.571 (88%)		$\times 10^3/\mu L$
Lymph	0.339 (3%)	L	$\times 10^3/\mu L$
Mono	0.678 (6%)		$\times 10^3/\mu L$
Eos	0.339 (3%)		$\times 10^3/\mu L$
Baso	0.113 (1%)		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	clear		
Sp Gr	1.033		
pH	6.5		
Protein	negative		
Other chemistries	negative		
Sediment	none		
	Serum Chemistry		
BUN	14		mg/dL
Creatinine	1.2		mg/dL
Total protein	3.21	L	g/dL
Albumin	1.4	L	g/dL
A/G ratio	0.78		

Free ebooks ==> www.Ebook777.com

ALP	102		U/L
ALT	42		U/L
Glucose	70	L	mg/dL
Sodium	145		mmol/L
Potassium	4.5		mmol/L
Chloride	121		mmol/L
Total CO_2	19		mmol/L
Anion gap	9.5		mmol/L
Calcium	8.4	L	mg/dL
	Other Tests		
TLI	15.5		µg/L
Feces:			
Trypsin	present		
Fat (direct)	1+		
Fat (acid + heat)	3+		
Parasites	negative		
Fat absorption:			
Prefeeding	plasma clear		
2-hour postfeeding	plasma clear		
3-hour postfeeding	plasma clear		
D-xylose absorption:			
Fasting	1.2		mg/dL
30 min	18.2		mg/dL
60 min	23.4	L	mg/dL
90 min	29.1	L	mg/dL
120 min	30.2	L	mg/dL
180 min	27.1		mg/dL

PROBLEMS:

1. **Malabsorption**.
   - **a.** The fecal fat screening tests suggest the presence of **steatorrhea** composed predominately of split fats. With the addition of acetic acid and heat, the free fatty acids and glycerol are recombined into triglyceride that can be identified as red-orange droplets with Sudan III stain.
   - **b.** **Absence of fat absorption** suggests malabsorption because the predominance of split fats in the feces suggests that pancreatic lipase is being produced.
   - **c.** **Deficient d-xylose absorption** (peak value <45 mg/dl) further suggests malabsorption. D-xylose is a simple sugar that should be readily absorbed and appear in the blood.
   - **d.** The presence of **protease activity** in fecal screening tests and a serum **TLI concentration within the reference interval** indicates a functional exocrine pancreas. Therefore, the laboratory abnormalities are due to an absorptive problem rather than digestive problem.

2. **Hypoproteinemia, hypoalbuminemia, and A/G ratio within the reference interval**. The hypoproteinemia is characterized by **hypoalbuminemia**, a calculated **hypoglobulinemia**, and an **A/G ratio within the reference interval**. Loss of albumin and globulins concurrently indicate **panhypoproteinemia**. Clinical evidence of hemorrhage, liver disease, and proteinuria are not present to explain the hypoproteinemia. Therefore, signs of intestinal disease suggest malabsorption of protein and/or enteric protein loss.

www.Ebook777.com

3. **Hypoglycemia**. Malabsorption of carbohydrate may lead to decreased blood glucose concentration.

4. **Hypocalcemia**. The correction formula for hypoproteinemia (8.4 to 0.4[3.21] + 3.3 = 10.9) adjusts the calcium concentration into the reference interval, indicating that hypoalbuminemia is responsible for the decreased serum calcium concentration. In health, approximately 40% of serum calcium is bound to proteins, especially albumin. Measurement of total calcium via a colorimetric method will often result in hypocalcemia when hypoalbuminemia is present. Signs of hypocalcemia are not present because the ionized (biologically active) fraction of calcium is most likely within the reference interval.

5. **Lymphopenia**. Loss of intestinal lymph, which is lymphocyte-rich afferent lymph, into the lumen of the intestine can lead to lymphopenia concomitant with intestinal protein loss.

SUMMARY: Intestinal biopsies revealed inflammatory bowel disease. Severe inflammatory bowel disease may be associated with protein-losing enteropathy, lymphocyte loss into the intestinal lumen, and malabsorption of fat. Another mechanism, such as intestinal bacterial overgrowth, apparently is responsible for the abnormal d-xylose absorption because simple sugars are absorbed into the portal blood rather than into lymphatics.

Free ebooks ==> www.Ebook777.com

CASE 17 MUSCLE DISEASE, MYOGLOBINURIC NEPHROSIS

SIGNALMENT: Equine, Quarterhorse, mare (female), 5 years old

PRESENTING PROBLEMS: Profuse sweating, pyrexia, stiffness, pain on palpation of firm gluteal muscles, oliguria

LABORATORY DATA:

	Hematology		
Plasma color: light yellow			
Hct	37		%
Hb	13		g/dL
RBC morphology: normal			
Platelets: adequate			
WBC	16.1	H	$\times 10^3/\mu L$
Seg	11.916 (74%)	H	$\times 10^3/\mu L$
Band	0.483 (3%)	H	$\times 10^3/\mu L$
Lymph	2.415 (15%)		$\times 10^3/\mu L$
Mono	1.288 (8%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
Fibrinogen	850	H	mg/dL
	Serum Chemistry		
BUN	70	H	mg/dL
Creatinine	4.7	H	mg/dL
Total protein	6.6		g/dL
Albumin	2.9		g/dL
A/G ratio	0.78		
AST	960	H	U/L
CK	640	H	U/L
Glucose	90		mg/dL
Calcium	11.2		mg/dL
Phosphorus	5.4	H	mg/dL

continued

www.Ebook777.com

	Urinalysis
Urine source	catheterization
Color	brown
Turbidity	cloudy
Sp Gr	1.020
pH	7.5
Protein	2+
Glucose	negative
Ketone	negative
Bilirubin	negative
Occult blood	4+
Sediment	0 to 1 RBC/HPF
	4 to 5 WBC/HPF
	2 to 3 granular casts/HPF
	$CaCO_3$ crystals

PROBLEMS:

1. **Leukocytosis**, **neutrophilia**, **left shift**, and **monocytosis** are consistent with an **inflammatory leukogram**.

a. **Neutrophilia** indicates a tissue demand for phagocytes.

b. Left shifts are more subtle in large animals and indicate that the maturation and storage pool of neutrophils in the bone marrow is being depleted of segmenters. Neutrophil release from the bone marrow into the blood is an age-ordered process.

c. Monocytosis may occur in acute and chronic disease. In this horse, monocytosis probably indicates a tissue demand for phagocytosis. Blood monocytes provide a replacement pool for tissue phagocytes.

2. **Hyperfibrinogenemia.** Fibrinogen is a positive acute-phase reactant because its concentration increases during inflammation. Fibrinogen is commonly determined as part of the CBC count in large animals because it may be an early indicator of inflammation, even when changes in the leukogram are not observed.

3. **Myoglobinuria**.

a. **Brown to red-brown discoloration of urine** can occur with hematuria, hemoglobinuria, and myoglobinuria. Hematuria is excluded because significant numbers of erythrocytes are not present in the urine sediment. Hemoglobinuria may be accompanied by red discoloration of the plasma. The light yellow color of the plasma in this horse is probably due to β-carotenes from ingested forage. Myoglobinuria is unassociated with discoloration of the plasma. Myoglobin is one-fourth the size of hemoglobin and is rapidly filtered from the plasma into the urine. An absence of erythrocytes in urine sediment and lack of red discoloration of plasma suggest that **myoglobinuria** is present.

b. **Positive reaction for occult blood** on urine reagent strips may occur with intact erythrocytes, hemoglobin, and myoglobin, all of which have peroxidase activity.

c. **Proteinuria** is present. A positive protein test on urine reagent strips can be observed with excess renal loss of albumin, as well as with inflammation, hemoglobinuria, or myoglobinuria. Urinary sediment findings exclude hematuria and pyuria (inflammation). The probability of hemoglobinuria is excluded above.

Free ebooks ==> www.Ebook777.com

4. **Muscle disease**.
   a. **Increased AST activity**. AST is a cytosolic enzyme that is present in many cell types. Increased AST activity often accompanies liver disease, muscle disease, and *in vivo* or *in vitro* hemolysis. Hemolysis is excluded largely by the lack of red discoloration of the plasma and, presumably, in the serum specimen submitted for biochemical testing. Hepatocellular injury can be excluded by SDH testing. Myolysis or myonecrosis is a distinct possibility for increased AST activity in this horse.
   b. **Increased CK activity** indicates muscle damage. CK activity in serum is relatively specific for striated muscle injury in adult animals. Sequential determination of CK activity will reveal if the muscle disorder is persistent, progressive, or resolving.

5. **Renal failure**.
   a. **Azotemia** can result from prerenal, renal, and post-renal causes. If sweating is extreme and water intake is restricted, this horse could be dehydrated. However, dehydration was not observed during the physical examination. The urine specimen was obtained by catheterization and post-renal obstruction was excluded. Therefore, the azotemia is probably of renal origin.
   b. **Low urine Sp Gr is present**, but has not reached the isosthenuric range (1.008 to 1.012).
   c. **Hyperphosphatemia** suggests a decreased glomerular filtration rate, probably a consequence of renal failure. However, muscle necrosis also can be associated with hyperphosphatemia.
   d. **Granular casts** indicate tubular disease of undetermined severity.

SUMMARY: The history, clinical signs, and laboratory findings support a diagnosis of primary muscle disease with rhabdomyolysis, secondary myoglobinuric nephrosis, and renal failure.

www.Ebook777.com

CASE 18 ACUTE RENAL FAILURE (ETHYLENE GLYCOL TOXICOSIS)

SIGNALMENT: Canine, Labrador Retriever, female, 1 year old

PRESENTING PROBLEMS: Lethargy, depression, ataxia, vomiting, dehydration, oliguria

LABORATORY DATA:

	Hematology		
Hct	56.5	H	%
Hb	18.6	H	g/dL
RBC	7.93	H	$\times 10^6/\mu L$
MCV	71.3		fL
MCH	23.5		pg
MCHC	32.9		%
RBC morphology: normal			
Platelets	357		$\times 10^3/\mu L$
WBC	29.7	H	$\times 10^3/\mu L$
Seg	26.433 (89%)	H	$\times 10^3/\mu L$
Band	2.079 (7%)	H	$\times 10^3/Ll$
Lymph	0.594 (2%)		$\times 10^3/\mu L$
Mono	0.594 (2%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	cystocentesis		
Color	light yellow		
Turbidity	clear		
Sp Gr	1.011		
pH	6.0		

Free ebooks ==> www.Ebook777.com

Protein	1+		
Glucose	1+		
Ketone	negative		
Bilirubin	negative		
Blood	negative		
Sediment	calcium oxalate monohydrate crystals		
	Serum Chemistry		
BUN	75	H	mg/dL
Creatinine	5.7	H	mg/dL
Total protein	8.2	H	g/dL
Albumin	3.1		g/dL
A/G ratio	0.61		
ALP	83		U/L
ALT	28		U/L
Glucose	141	H	mg/dL
Sodium	143		mmol/L
Potassium	5.5	H	mmol/L
Chloride	99	L	mmol/L
Total CO_2	5	L	mmol/L
Anion gap	39	H	mmol/L
Calcium	8.6	L	mg/dL
Phosphorus	10.2	H	mg/dL
	Blood Gases		
pH	7.237	L	
HCO_3	11.5	L	mmol/L
PCO_2	27.1	L	mmHg

PROBLEMS:

1. **Renal failure**. **Isosthenuria** in the presence of **azotemia** indicates renal failure. **Hyperphosphatemia** reflects the decreased glomerular filtration rate. The rust inhibitor in antifreeze also is a source of phosphate.

2. **Metabolic acidosis with partial respiratory compensation**. This problem is indicated by a **decreased blood pH** (acidemia), **decreased bicarbonate concentration**, and **decreased pCO_2**. The **high anion gap** indicates a titration acidosis. Metabolites of ethylene glycol and uremic acids are responsible for the increased anion gap.

3. **Polycythemia and hyperproteinemia**. Dehydration causes a relative **hyperproteinemia** and the **A/G ratio remains within the reference interval**. The **high-normal hematocrit** also may be due to dehydration.

4. **Calcium oxalate monohydrate crystalluria**. This finding is highly suggestive of ethylene glycol toxicosis.

5. **Proteinuria**. Mild proteinuria, in the absence of hematuria or pyuria, indicates renal origin. Mild proteinuria can occur in tubular disease.

6. **Mild hyperglycemia**. Catecholamine release is probably responsible for the hyperglycemia. Previous blood glucose values would have to exceed 180 mg/dl to produce **glucosuria**. In addition, massive renal

www.Ebook777.com

tubular nephrosis from crystal deposition could cause acute renal disease with subsequent mild glucosuria. If many renal tubular epithelial cells are destroyed in acute ethylene glycol toxicosis, glucose will not be reclaimed from the glomerular filtrate and mild glucosuria will occur in the presence of normoglycemia or mild hyperglycemia that does not exceed the renal threshold.

7. **Normonatremic dehydration**, **hypochloridemia**, and **hyperkalemia**. There is a balanced loss of water and Na^+ ions. **Hypochloridemia** may be the result of vomiting. Acidemia is causing a shift of K^+ to the extracellular fluid in exchange for hydrogen ions. Oliguria is preventing excretion of excess K^+, although K^+ loss in vomitus is likely. The balance of these changes has resulted in hyperkalemia at this time.

8. **Hypocalcemia**. Renal failure can cause hypocalcemia via several mechanisms; serum calcium concentration also may be decreased in this dog because of formation of calcium oxalate crystals.

9. **Inflammatory leukogram**. **Neutrophilia** with a **left shift** suggests clinically important inflammation.

SUMMARY: Oxalate nephrosis was diagnosed at necropsy. Ethylene glycol toxicosis may cause renal failure, a high anion gap, hypocalcemia, and calcium oxalate monohydrate crystalluria. Depending upon the degree of renal tubular nephrosis, mild glucosuria may occur in the presence of normoglycemia or mild hyperglycemia. A plasma test is available that will confirm early ethylene glycol toxicosis so treatment can be instituted before nephrosis and crystalluria develop. Propylene glycol (a food preservative) or glycerol can cause a false positive test result with this assay. A false negative test result may occur if blood samples are obtained >12 hours after ingestion of ethylene glycol because it will have been converted into other toxic metabolites.

Free ebooks ==> www.Ebook777.com

CASE 19 RENAL AMYLOIDOSIS (NEPHROTIC SYNDROME)

SIGNALMENT: Dog, Pointer, male, 6 years old

PRESENTING PROBLEMS: Vomiting, PU/PD, dependent edema, ascites

LABORATORY DATA:

	Hematology		
Hct	18	L	%
Hb	6.3	L	g/dL
RBC	2.51	L	$\times 10^6/\mu L$
MCV	71		fL
MCH	20.6		pg
MCHC	35.0		%
Retic	0		%
Abs retic	0		$\times 10^3/\mu L$
RBC morphology: normal			
Platelets: adequate			
WBC	20.1	H	$\times 10^3/\mu L$
Seg	18.894 (94.0%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	0.704 (3.5%)		$\times 10^3/\mu L$
Mono	0.301 (1.5%)		$\times 10^3/\mu L$
Eos	0.201 (1.0%)		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	cloudy		
Sp Gr	1.016		
pH	7.0		
Protein	4+		
Glucose	negative		
Ketone	negative		

continued

www.Ebook777.com

Bilirubin	negative		
Blood	negative		
Sediment	1 to 2 RBC/hpf 2 to 5 WBC/hpf granular casts amorphous crystals		
	Chemistry		
BUN	133	H	mg/dL
Creatinine	4.1	H	mg/dL
Total protein	3.3	L	g/dL
Albumin	0.7	L	g/dL
A/G ratio	0.28	L	
ALP	43		U/L
ALT	21		U/L
Glucose	112		mg/dL
Sodium	151		mmol/L
Potassium	5.1		mmol/L
Chloride	124		mmol/L
Total CO_2	17		mmol/L
Anion gap	15.1		mmol/L
Calcium	8.4	L	mg/dL
Phosphorus	6.9	H	mg/dL
Cholesterol	530	H	mg/dL

Other Tests

Abdominal fluid analysis:
- Clear and colorless
- Nucleated cell count = 200 cells/µl
- Nondegenerate neutrophils, macrophages, mesothelial cells
- Protein = < 2.5 g/dL
- Opinion: transudate

Urine protein/urine creatinine ratio = 12.2

PROBLEMS:

1. Anemia of chronic renal disease. **Normocytic**, **normochromic**, **nonregenerative anemia** accompanying the azotemia is usually a consequence of chronic renal disease (see below) with the loss of erythropoietin-secreting peritubular cells.

2. Stress leukogram. A **neutrophilia without a left shift** and a **low-normal lymphocyte count** suggest systemic stress. Similar changes in the leukogram also may be seen in chronic inflammation.

3. Possible early renal failure. Azotemia and a low urine Sp Gr are suggestive of decreased renal concentrating ability or possible early renal failure. Marked proteinuria also is present (see below).

a. Azotemia can be of prerenal, renal, or postrenal origin. Postrenal azotemia is excluded because the urine specimen is voided. Vomiting may contribute to prerenal azotemia, but dehydration was not noted in the physical findings. Therefore, azotemia is probably of renal origin.

b. Low urine Sp Gr is concomitant with **polyuria** and **polydypsia**. Lack of renal concentrating ability results in polyuria and subsequent polydypsia. With primary glomerular lesions (e.g., renal amyloidosis, glomerulonephritis), azotemia may precede the loss of urine concentrating ability. This

Free ebooks ==> www.Ebook777.com

pattern has been called glomerulotubular imbalance because tubular dysfunction is usually the first abnormality in renal failure. Fluid loss occurs with vomiting. Therefore, the urine should be more concentrated to conserve body water unless the vomiting is of very recent onset.

4. **Renal proteinuria**. **4+ proteinuria** without significant cellular sediment and a negative reaction for occult blood (which excludes hematuria, hemoglobinuria, and myoglobinuria) denotes proteinuria of renal origin. Marked albumin loss can be expected with this magnitude of proteinuria in dilute urine. A **urine protein/urine creatinine ratio > 3** suggests that the proteinuria is of glomerular origin. The highest urine protein/urine creatinine ratios are observed with renal amyloidosis.

5. **Hypoproteinemia**, **hypoalbuminemia**, **hypoglobulinemia**, and **decreased A/G ratio**. **Hypoproteinemia** is primarily the result of albumin loss in the urine. A **decreased A/G ratio** usually occurs with hypoalbuminemia and/or hyperglobulinemia. In this patient, **hypoglobulinemia** is present, presumably from renal loss. However, a proportionally greater quantity of albumin is being lost in the urine. Glomerular amyloid deposition initially causes selective loss of albumin, but as the disease progresses, larger protein molecules (globulins) also may be lost via the urine.

6. **Hypocalcemia**. **Hypocalcemia** is a reflection of **hypoalbuminemia**. In the serum, 50% of the calcium is in the ionized form, 40% is bound to protein, especially albumin, and 10% is complexed with anions such as phosphate and citrate. Therefore, hypoalbuminemia can result in significant hypocalcemia if total calcium is determined by a colorimetric spectrophotometric technique. Clinical signs of hypocalcemia will not be apparent unless the ionized calcium concentration decreases. Using a mathematical formula to adjust the serum calcium concentration for the effect of hypoalbuminemia results in normocalcemia (10.4 mg/dl). Hypocalcemia occasionally may be observed with renal failure.

7. **Hyperphosphatemia**. **Hyperphosphatemia** is observed frequently in dogs with renal disease. This laboratory abnormality reflects a decreased glomerular filtration rate.

8. **Hypercholesterolemia**. Cholesterol synthesis is thought to be stimulated by the reduced osmotic pressure caused by hypoalbuminemia.

9. **Low-protein ascites and edema**. Severe **hypoalbuminemia** results in **decreased plasma oncotic pressure** with development of a **low-protein ascites** and/or **edema**. The edema may be generalized (anasarca), but gravitational influences may cause the edema to be most visible in the ventrum and extremities (dependent edema).

SUMMARY: Renal amyloidosis was diagnosed by renal biopsy. The tetrad of edema, hypoproteinemia, proteinuria, and hypercholesterolemia characterizes the nephrotic syndrome, which is caused by primary glomerular disease.

www.Ebook777.com

CASE 20 FELINE LOWER URINARY TRACT DISEASE (FELINE UROLOGIC SYNDROME)

SIGNALMENT: Cat, domestic shorthair, male, 3 years old

PRESENTING PROBLEMS: Anorexia, depression, vomiting, unable to urinate, tense, enlarged abdomen

LABORATORY DATA:

	Hematology		
Hct	39		%
RBC morphology: normal			
Platelets: adequate			
WBC	18.4	H	$\times 10^3/\mu L$
Seg	16.928 (92%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	1.452 (8%)	L	$\times 10^3/\mu L$
Mono	0.0		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	cystocentesis		
Color	reddish-brown		
Turbidity	cloudy		
Sp Gr	1.031		
pH	7.5		
Protein	3+		
Glucose	negative		
Ketone	negative		
Bilirubin	negative		
Blood	4+		
Sediment	innumerable RBC		
	15 to 20 WBC/hpf		
	triple phosphate crystals		
	3+ bacterial rods		

Free ebooks ==> www.Ebook777.com

	Chemistry		
BUN	169	H	mg/dL
Creatinine	13.6	H	mg/dL
Total protein	6.9		g/dL
Albumin	2.9		g/dL
A/G ratio	0.73		
ALP	22		U/L
ALT	26		U/L
Glucose	175	H	mg/dL
Sodium	138	L	mmol/L
Potassium	7.9	H	mmol/L
Chloride	102	L	mmol/L
Total CO_2	10	L	mmol/L
Anion gap	30.9	H	mmol/L
Calcium	10.1		mg/dL
Phosphorus	11.2	H	mg/dL

PROBLEMS:

1. **Stress leukogram**. **Neutrophilia** without a significant left shift, **lymphopenia**, and **eosinopenia** denote endogenous corticosteroid release secondary to stress (painful disorder).

2. **Hemorrhagic cystitis and urinary tract infection**.
   a. **Positive reagent strip (dipstick) reactions for urine protein and occult blood** associated with significant erythrocytes in the sediment indicate **hematuria.**
   b. **Pyuria** also is present based on urine sediment examination (15 to 20 WBC/hpf). Thus, the **proteinuria** is the result of the hemorrhage and inflammation.
   c. **Bacteriuria**. Collection of urine by cystocentesis eliminates the genital tract as the source of the hemorrhage, leukocytes, and **bacteriuria. Urinary tract infection** is confirmed by the presence of bacteria.

3. **Postrenal azotemia**. Clinical evidence of urinary tract obstruction is necessary to diagnose postrenal azotemia. Renal concentrating abnormalities may or may not occur with urinary obstruction.

4. **Hyperglycemia**. The **hyperglycemia** may be a manifestation of endogenous cortisol and/or catecholamine release.

5. **Metabolic acidosis**, **high anion gap**, **hyponatremia**, **hypochloridemia**, and **hyperkalemia**.
   a. **Metabolic acidosis** is caused by titration of bicarbonate by uremic acids.
   b. The **high anion gap** is due to unmeasured anions. Uremic acids are probably responsible for this change; however, lactic acid accumulation from shock also could be possible.
   c. **Hyponatremia** with clinically normal hydration indicates **low total body Na^+** concentration. Vomiting will result in Cl^- loss, causing **hypochloridemia**.
   d. **Hyperkalemia** is a sequel to anuria because excess K^+ is eliminated from the body in the urine. Acidosis also may be a factor (see below).
   e. The **decreased TCO_2 concentration** indicates **acidosis**. Acidosis is a complicating factor in hyperkalemia because it causes a shift of K^+ to the extracellular fluid in exchange for hydrogen ions that move into the intracellular fluid.

6. **Hyperphosphatemia** is further evidence of reduced glomerular filtration.

SUMMARY: Catheterization dislodged a urethral calculus. The life-threatening hyperkalemia and other laboratory abnormalities subsided with supportive treatment.

www.Ebook777.com

CASE 21 ACUTE SEPTIC MASTITIS

SIGNALMENT: Bovine, Holstein, cow (female), 6 years old

PRESENTING PROBLEMS: Hot swollen mammary gland quarter of less than 12 hours duration with yellowish watery milk, pyrexia, anorexia

LABORATORY DATA:

	Hematology		
Hct	37		%
Hb	12.2		g/dL
RBC morphology: normal			
Platelets: adequate			
WBC	5.9		$\times 10^3/\mu L$
Seg	0.708 (12%)	L	$\times 10^3/\mu L$
Band	1.829 (31%)	H	$\times 10^3/\mu L$
Other	0.177 (3%)	H	$\times 10^3/\mu L$
Lymph	2.891 (49%)		$\times 10^3/\mu L$
Mono	0.295 (5%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: cytoplasmic basophilia and vacuolation			
Fibrinogen	1400	H	mg/dL
	Serum Chemistry		
BUN	21		mg/dL
Total protein	7.6		g/dL
Albumin	3.2		g/dL
A/G ratio	0.73		
AST	210	H	U/L
Glucose	222	H	mg/dL
Sodium	151		mmol/L
Potassium	4.2		mmol/L
Chloride	102		mmol/L
Total CO_2	22		mmol/L
Anion gap	31.2	H	mmol/L

Free ebooks ==> www.Ebook777.com

PROBLEMS:

1. **Peracute purulent inflammatory disease**.
   a. **Neutropenia**, **degenerative left shift**, and **toxic changes of neutrophils** indicate peracute inflammatory disease in cattle. The degenerative left shift indicates that the bone marrow is failing to meet tissue demands for phagocytes and that the maturation and storage pool of segmented neutrophils in the marrow is exhausted. Sequential leukograms revealing an increasing neutrophil count would indicate a favorable response to the tissue demand for phagocytes; whereas persistent or intensifying neutropenia would denote a guarded to poor prognosis. **Toxic changes of neutrophils** reflect severe bacterial infection and toxemia in this cow.
   b. **Hyperfibrinogenemia** also indicates peracute inflammatory disease. The concentration of this acute-phase reactant increases in inflammation. Hyperfibrinogenemia may indicate the presence of inflammation in cattle before changes are apparent in the leukogram.

2. **Increased AST activity**. Many cell types exhibit AST activity. Increased enzymatic activity in serum commonly accompanies **hepatocellular disease**, **muscle injury**, and *in vivo* or *in vitro* **hemolysis**. There is no mention of hemolysis in the plasma (for the CBC count) or in the serum (for biochemical analysis). Therefore, increased AST activity may indicate hepatocellular or muscle disease. Increased **SDH** activity would confirm hepatic disease, while increased **CK** activity would confirm muscle injury.

3. **Hyperglycemia**. Hyperglycemia probably indicates endogenous catecholamine release in this cow.

4. **High anion gap**. The high anion gap indicates an increased concentration of unmeasured anions. Lactate production may occur secondary to shock. Ketosis also can result in an increased anion gap and may be detected by reagent strip testing during routine urinalysis. Both ketoacids and lactic acid will decrease plasma bicarbonate (TCO_2) by titration. Because TCO_2 is within the reference interval, a concomitant alkalosis (due to abomasal reflux) is suspected, causing a mixed metabolic acidosis and alkalosis. Blood gas analysis will better define the acid-base imbalance.

SUMMARY: Acute septic mastitis was diagnosed clinically. A guarded prognosis was based on the initial laboratory data. Although electrolyte imbalance was not suspected clinically, the increased anion gap revealed an otherwise occult acidosis.

www.Ebook777.com

CASE 22 NEPHROSIS AND PERIRENAL HEMORRHAGE

SIGNALMENT: Bovine, Hereford, cow (female), 12 years old

PRESENTING PROBLEMS: Hematuria, dehydration estimated clinically at 8%

LABORATORY DATA:

	Hematology		
Hct	30		%
RBC morphology: normal			
Platelets: increased			
WBC	9.338		$\times 10^3/\mu L$
Seg	7.740 (80%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	1.307 (14%)	L	$\times 10^3/\mu L$
Mono	0.373 (4%)		$\times 10^3/\mu L$
Eos	0.093 (1%)		$\times 10^3/\mu L$
Baso	0.093 (1%)		$\times 10^3/\mu L$
WBC morphology: normal			
Fibrinogen	1800	H	mg/dL
	Urinalysis		
Urine source	voided		
Color	red		
Turbidity	cloudy		
Sp Gr	1.018		
pH	7.0		
Protein	3+		
Glucose	2+		
Ketone	negative		
Bilirubin	negative		
Blood	4+		
Sediment	innumerable RBC/hpf 1 to 2 granular casts/hpf		
	Chemistry		
BUN	161	H	mg/dL
Creatinine	10.5	H	mg/dL

Free ebooks ==> www.Ebook777.com

Total protein	8.2	H	g/dL
Albumin	3.1	H	g/dL
A/G ratio	0.61		
AST	97		U/L
Glucose	498	H	mg/dL
Sodium	115	L	mmol/L
Potassium	2.2	L	mmol/L
Chloride	50	L	mmol/L
Total CO_2	38.3	H	mmol/L
Anion gap	28.9	H	mmol/L
Calcium	7.3	L	mg/dL
Phosphorus	8.5	H	mg/dL
	Other Tests		
Blood gas analysis (venous):			
HCO_3^-	37.1	H	mmol/L
PCO_2	37.7		mmHg
pH	7.483	H	

PROBLEMS:

1. **Systemic stress and possible inflammation**. Although the leukocyte count is within the reference interval, **neutrophilia** and **lymphopenia** are present. The neutrophilia suggests either systemic stress or mild inflammation. Lymphopenia is commonly observed in stress. Endogenous cortisol release can cause these changes; however, early inflammation also is a possibility.

2. **Hyperfibrinogenemia**. Hyperfibrinogenemia indicates inflammation. Hyperfibrinogenemia is an acute-phase reactant whose concentration in the plasma increases during inflammation. Marked hyperfibrinogenemia, measured by the heat precipitation test, is observed commonly in bovine renal disease as well as inflammatory conditions. Hyperfibrinogenemia may indicate early inflammation in cattle and precedes changes in the leukogram.

3. **Thrombocytosis**. Reactive thrombocytosis commonly accompanies hemorrhage. Assessment of thrombocytosis is subjective and based on examination of the stained blood smear. More accurate thrombocyte data can be provided by the platelet count and determination of the MPV.

4. **Renal disease**.
   a. **Azotemia** may be of prerenal, renal, or post-renal origin. Because the urine specimen is voided, post-renal azotemia is excluded. Dehydration may have contributed to azotemia; however, the urine Sp Gr is low. Therefore, **low urine Sp Gr** with **8% dehydration** suggests renal failure.
   b. **Proteinuria** and **hematuria** may be associated with a renal lesion or lower urinary tract problem. **Granular casts** indicate a tubular lesion of unknown severity.

5. **Hyperproteinemia**, **hyperalbuminemia**, **A/G ratio is within the reference interval.** By mathematical calculation, **hyperglobulinemia** also is present. The normal A/G ratio indicates that the concentrations of albumin and globulins are proportionally increased. This change is secondary to dehydration and plasma water loss. The presence of 8% dehydration is important because the rumen contains a large fluid reserve; thus, dehydration in this animal is severe.

6. **Hyperglycemia with glucosuria. Hyperglycemia** may be observed in extremely ill or moribund cattle. Catecholamine and cortisol release probably play a role in development of hyperglycemia, but diabetes mellitus cannot be excluded with certainty. The bovine renal threshold for glucose is 100 mg/

www.Ebook777.com

dl. Because serum glucose concentration has exceeded this threshold, glucosuria has occurred. Solute diuresis may further enhance the dehydration.

7. **Hyponatremic dehydration and hypokalemia**. Bovine renal disease causes urinary loss of Na^+ and water in excess of fluid and electrolyte intake. Total body Na^+ content is very low. Hypokalemia is probably secondary to changes in external K^+ balance (i.e., decreased oral K^+ intake and kaliuresis) and alkalosis in which K^+ shifts from ECF to ICF.

8. **Mixed metabolic alkalosis and acidosis, hypochloridemia, and increased anion gap**.

a. **High plasma TCO_2 and HCO_3^- concentration** (metabolic alkalosis) and **hypochloridemia** are common in bovine renal disease due to upper gastrointestinal stasis and functional loss of abomasal HCl.

b. The blood gas analysis confirms alkalemia. The pCO_2 is within the reference interval, indicating that respiratory compensation has not occurred.

c. The **high anion gap** suggests a concurrent metabolic acidosis caused by titration of plasma buffer systems with uremic acids, phosphoric acid (high P concentration), and perhaps lactic acid (shock). At this time, the balance between these two abnormalities has resulted in an alkalemia.

9. **Hypocalcemia** and **hyperphosphatemia**. Hypocalcemia is frequently observed in bovine renal disease. Hyperphosphatemia may occur with a decreased GFR from dehydration and renal disease.

SUMMARY: Following necropsy and histopathology, unilateral perirenal and renal hemorrhage and bilateral nephrosis were diagnosed. The etiology of these lesions was not determined.

Free ebooks ==> www.Ebook777.com

CASE 23 RIGHT ABOMASAL DISPLACEMENT

SIGNALMENT: Bovine, Jersey, cow (female), 2 1/2 years old

PRESENTING PROBLEMS: Anorexia, decreased milk production, ruminal and intestinal atony, decreased respiratory rate, dehydration estimated clinically at 3%

LABORATORY DATA:

	Hematology		
Hct	36.7		%
Hb	12.6		g/dL
RBC	8.89		$\times 10^6/\mu L$
MCV	41.3		fL
MCH	13.6		pg
MCHC	34.3		%
RBC morphology: normal			
Platelets: adequate			
WBC	16.8	H	$\times 10^3/\mu L$
Seg	12.264 (73%)	H	$\times 10^3/\mu L$
Band	0.168 (1%)		$\times 10^3/\mu L$
Lymph	2.856 (17%)		$\times 10^3/\mu L$
Mono	1.512 (9%)		$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
Fibrinogen	700	H	mg/dL
	Urinalysis		
Urine source	voided		
Color	pale yellow		
Turbidity	clear		
Sp Gr	1.012		
pH	5.0		
Protein	negative		
Glucose	2+		

continued

www.Ebook777.com

Ketone	negative		
Bilirubin	negative		
Blood	negative		
Sediment	none		
	Chemistry		
Creatinine	2.1		mg/dL
Glucose	231	H	mg/dL
Sodium	136		mmol/L
Potassium	3.1	L	mmol/L
Chloride	86	L	mmol/L
Total CO_2	40.0	H	mmol/L
Anion gap	13.1		mmol/L
Calcium	8.6		mg/dL
	Other Tests		
Blood gas analysis (venous):			
HCO_3^-	38.4	H	mmol/L
PCO_2	52.6	H	mmHg
pH	7.492	H	

PROBLEMS:

1. **Neutrophilic leukocytosis**. Neutrophilia without a left shift occurs in some noninflammatory conditions such as displaced abomasum. It is probably caused by mild inflammation or endogenous cortisol release.

2. **Hyperfibrinogenemia**. Hyperfibrinogenemia indicates inflammation and suggests that the changes in the leukogram are not merely the result of stress. Hyperfibrinogenemia may indicate early inflammation in cattle and precede changes in the leukogram.

3. **Hyperglycemia and glucosuria**. Hyperglycemia in cattle generally is transient and may be induced by endogenous catecholamine or corticosteroid release. During transient hyperglycemia, blood glucose concentration may exceed the bovine renal threshold (100 mg/dL) in nondiabetic conditions and glucosuria will occur.

4. **Metabolic alkalosis, hypochloridemia, and paradoxical aciduria**. Abomasal displacement causes sequestration and loss of gastric HCl to reabsorption with a net gain in HCO_3^- (**increased TCO_2 and HCO_3**, alkalosis), and hypochloridemia. A decreased respiratory rate compensates for the alkalosis and **PCO_2 increases** in an effort to restore HCO_3^-/H_2CO_3 ratio to 20:1 (ratio = approximately 24:1 in this case). Renal correction of metabolic alkalosis normally involves secretion of excess HCO_3^- and retention of H^+ ions. HCO_3^- is reabsorbed instead of Cl^- because of a deficiency of Cl^- and K^+ in the plasma and glomerular filtrate. As the kidney reabsorbs Na^+ to restore total body water, H^+ is secreted instead of K^+. This potentiates the alkalosis and causes paradoxical aciduria. The **anion gap is within the reference interval**, excluding a complicating titration-type metabolic acidosis in this cow.

5. **Hypokalemia**. Anorectic herbivores often have negative K^+ balance due to decreased oral electrolyte intake and continued renal electrolyte loss. In addition, loss of gastric and intestinal fluids to reabsorption (as in abomasal displacement) represents an additional change in external K^+ balance. Internal shift of K^+ ions, in which ECF K^+ is exchanged for ICF H^+ during alkalosis, is thought to be a minor cause of hypokalemia.

SUMMARY: Right-sided abomasal displacement was confirmed by laparotomy and surgically corrected.

Free ebooks ==> www.Ebook777.com

CASE 24 ACUTE PULMONARY HEMORRHAGE AND EDEMA (ACUTE PARAQUAT® TOXICOSIS)

SIGNALMENT: Canine, German Shepherd, female, 2 years old

MEDICAL HISTORY: Known exposure to Paraquat®. Continuous intravenous lactated Ringer's solution (4,700 ml total volume) spiked with KCl (totaling 39 mEq/L) was administered intravenously over a 20-hour period prior to obtaining blood and urine samples for laboratory evaluation.

PRESENTING PROBLEMS: Severe vomiting and rapid, deep, painful (groaning) respiration

LABORATORY DATA:

	Hematology		
Hct	52.5	H	%
	Urinalysis		
Urine source	catheterization		
Color	pale yellow		
Turbidity	clear		
Sp Gr	1.015		
pH	7.5		
Chemistries	negative		
Sediment	none		
	Serum Chemistry		
BUN	58	H	mg/dL
Glucose	120		mg/dL
Sodium	127	L	mmol/L
Potassium	2.8	L	mmol/L
Chloride	78	L	mmol/L
Total CO_2	26.8	H	mmol/L
Anion gap	25.0	H	mmol/L
	Other Tests		
Blood gas analysis (arterial):			
HCO_3^-	25.5	H	mmol/L
PCO_2	32.8		mmHg
pH	7.515	H	
PO_2	52.0	L	mmHg

www.Ebook777.com

PROBLEMS:

1. **Polycythemia**. Polycythemia is present even after a history of fluid therapy. Relative polycythemia is probably the result of dehydration from severe vomiting. Vomiting animals often have decreased oral water intake. Absolute polycythemia can be caused by chronic hypoxia from high altitude, left to right sided cardiac shunts, lung disease, renal cysts or neoplasms (paraneoplastic syndrome), or polycythemia vera, a neoplastic condition in which unregulated production of mature erythrocytes occurs.

2. **Possible renal failure**.
   a. **Azotemia** can be prerenal, renal, or post-renal in origin. The presence of **polycythemia**, despite fluid therapy, suggests **dehydration** and possible prerenal azotemia.
   b. **Azotemia and low urine specific gravity** indicate **possible renal failure**. However, the low urine Sp Gr may represent diuresis secondary to fluid therapy.

3. **Hyponatremia, hypokalemia, and hypochloridemia**. Because dehydration was not clinically apparent at the time of sampling, ECF volume is presumed to be normal or increased (possible pulmonary edema). These data indicate that the Na^+ content of the intravenous fluids may have been inadequate and overhydration developed. Hypokalemia is a common sequela of prolonged fluid therapy, especially with alkalinizing fluid. During fluid administration, the ECF_{K+} shifts to the ICF in exchange for ICF_{H+}. Diuresis may produce kaliuresis, while alkalemia potentiates the internal shift of K^+ from ECF to ICF. Hypochloridemia parallels hyponatremia or may be a consequence of vomiting.

4. **Hypoxemia**. **Low arterial PO_2** concomitant with rapid respiration and **normal PCO_2** indicates a perfusion/diffusion abnormality as with pneumonia, pulmonary edema, or pulmonary thrombosis.

5. **Uncompensated mixed metabolic alkalosis and metabolic acidosis**. The marked **alkalemia** is due to a combination of mild metabolic alkalosis (**increased TCO_2** and **increased HCO_3^-**) and lack of respiratory compensation (normal PCO_2). The hypoxemia is maintaining the respiratory drive, preventing hypoventilation needed to increase PCO_2 to compensate for the metabolic alkalosis. The **high anion gap** suggests the possibility of a concomitant titration acidosis due to uremic acids or lactic acidosis. Administration of large quantities of lactated Ringer's solution also may increase the plasma lactate concentration and increase the anion gap. The high bicarbonate concentration could be due to bicarbonate addition to the intravenous fluids or conversion of lactate to bicarbonate.

SUMMARY: Diagnoses of nephrosis, pulmonary hemorrhage, and pulmonary edema were made following necropsy and histopathology. Paraquat® was verified on toxicologic analysis. Paraquat® is an herbicide that ultimately causes interstitial pneumonia, alveolar type II cell hyperplasia, and pulmonary fibrosis that compromise normal pulmonary function. These latter lesions develop only if sufficient time has elapsed between toxin ingestion and death.

Free ebooks ==> www.Ebook777.com

CASE 25 RODENTICIDE (COUMARIN) TOXICOSIS

SIGNALMENT: Canine, German Shepherd, male, 6 months old

MEDICAL HISTORY: A female littermate died recently with pulmonary hemorrhage.

PRESENTING PROBLEMS: Rear leg lameness, hyphema, and hematuria.

LABORATORY DATA:

	Hematology		
Hct	30.0	L	%
Hb	9.9	L	g/dL
RBC	4.16	L	$\times 10^6/\mu L$
MCV	72.1		fL
MCH	23.8		pg
MCHC	33.0		%
Retic	4.5	H	%
Abs retic	187	H	$\times 10^3/\mu L$
RBC morphology: polychromasia			
Platelets:	350		$\times 10^3/\mu L$
WBC	25.6	H	$\times 10^3/\mu L$
Seg	19.719 (77%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	1.792 (7%)		$\times 10^3/\mu L$
Mono	4.096 (16%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	voided		
Color	red		
Turbidity	cloudy		
Sp Gr	1.040		
pH	6.5		
Protein	4+		
Glucose	negative		
Ketone	negative		
Bilirubin	negative		

continued

www.Ebook777.com

Blood	4+		
Sediment	innumerable RBC/hpf 10 to 15 WBC/hpf		
	Serum Chemistry		
BUN	21		mg/dL
Creatinine	1.3		mg/dL
Total protein	5.2	L	g/dL
Albumin	2.5	L	g/dL
A/G ratio	0.93		
ALP	100		U/L
ALT	31		U/L
Glucose	111		mg/dL
Sodium	144		mmol/L
Potassium	4.0		mmol/L
Chloride	120		mmol/L
Total CO_2	15		mmol/L
Anion gap	10		mmol/L
	Other Tests		
Fibrinogen	256		mg/dL
ACT	290	H	sec
APTT	35.7	H	sec
PT	30.2	H	sec
TT	5.2		sec
FDP	<10		µg/mL

Synovial fluid analysis:
- red, cloudy, reduced viscosity
- poor mucin clot quality
- nucleated cell count: 1.3×10^6/µL
- numerous RBCs
- 20% neutrophils
- 80% monocytes and lymphocytes
- erythrophagcytosis by macrophages

Opinion: hemarthrosis

PROBLEMS:

1. **Normocytic, normochromic, regenerative anemia**. The **decreased hematocrit**, **hemoglobin**, and **RBC count** indicate **anemia**. The erythrocyte indices reveal that the anemia is **normocytic** and **normochromic**. The **absolute reticulocyte count** reveals erythrocyte **regeneration**. These laboratory changes and clinical evidence of bleeding are consistent with a hemorrhagic anemia.

2. **Leukocytosis, neutrophilia, monocytosis**. **Neutrophilia** and **monocytosis** commonly occur after hemorrhage.

3. **Hypoproteinemia, hypoalbuminemia, borderline hypoglobulinemia, A/G ratio within the reference interval**. The **hypoproteinemia** is the result of concurrent loss of albumin and globulins via hemorrhage. Because these proteins are lost together, the **A/G ratio remains within the reference interval**. The calculated globulin concentration is 2.7 g/dL, which is the low end of the reference interval. Thus, **borderline hypoglobulinemia** is present.

Free ebooks ==> www.Ebook777.com

4. **Coagulation factor deficiency, most likely an acquired vitamin K-dependent factor deficiency**. Hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency) are excluded because both the **APTT** and **PT are prolonged**; only the APTT wound be prolonged in these hereditary disorders. Von Willebrand's disease may have decreased factor VIII activity, but rarely is it low enough to prolong the APTT. **Normal platelet count**, **TT**, and **FDP** concentration, concomitant with the prolonged clotting times, tends to exclude acute uncompensated disseminated intravascular coagulation syndrome. **Prolonged ACT**, **APTT**, and **PT** are consistent with acquired deficiency of the vitamin K-dependent clotting factors (factors II, VII, IX, and X). Coumarin rodenticide toxicosis is highly likely. Liver disease, as a cause of acquired vitamin-K-dependent clotting factor deficiency, is unlikely because ALT and ALP activities are within the reference interval.

5. **Hematuria**. **Proteinuria** with a **positive reagent strip reaction for occult blood** and **erythrocytes in the urinary sediment** indicate hemorrhage.

6. **Hemarthrosis**. The presence of **numerous erythrocytes** and evidence of **erythrophagocytosis by macrophages** are consistent with hemarthrosis.

SUMMARY: Possible exposure to a coumarin-type rodenticide was confirmed by the owner. The dog was successfully treated with vitamin K_1 administration. Second generation rodenticides, such as diphacinone, have largely replaced first-generation coumarin products. Diphacinone is more toxic and persists longer; therefore, vitamin K_1 administration may have to be continued for 3 to 4 weeks to prevent the recurrence of bleeding.

www.Ebook777.com

CASE 26 HYPERADRENOCORTICISM

SIGNALMENT: Canine, Boston Terrier, castrated male, 13 years old

MEDICAL HISTORY: Previous corticosteroid therapy

PRESENTING PROBLEMS: Generalized alopecia, scaling, erythema, pyoderma, overweight, polyuria, polydipsia

LABORATORY DATA:

	Hematology		
Hct	48.8		%
Hb	16.6		g/dL
RBC	6.89		$\times 10^6/\mu L$
MCV	71		fL
MCH	24.1		pg
MCHC	34.0		%
RBC morphology: normal			
Platelets	468		$\times 10^3/\mu L$
WBC	30.3	H	$\times 10^3/\mu L$
Seg	27.573 (91%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	0.303 (1%)	L	$\times 10^3/\mu L$
Mono	2.424 (8%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	clear		
Sp Gr	1.006		
pH	7.0		
Chemistries	negative		
Sediment	squamous cells		

Free ebooks ==> www.Ebook777.com

	Chemistry		
BUN	26		mg/dL
Creatinine	0.7		mg/dL
Total protein	6.0		g/dL
Albumin	3.0		g/dL
A/G ratio	1.0		
ALP	1195	H	U/L
ALP post-levam	1102	H	U/L
ALP-levamisole resistance	92.2		%
ALT	482	H	U/L
Glucose	152	H	mg/dL
Sodium	140		mmol/L
Potassium	5.9		mmol/L
Chloride	113		mmol/L
Total CO_2	20		mmol/L
Anion gap	20		mmol/L
Calcium	10.2		mg/dL
	Other Tests		
ACTH stimulation test:			
pre-ACTH cortisol	2.16		
post-ACTH cortisol	21.14		
Low-dose dexamethasone suppression test:			
pre-dexamethasone cortisol	2.28		
8-hour post cortisol	1.78		

PROBLEMS:

1. **Leukocytosis, neutrophilia, lymphopenia, eosinopenia**. The leukogram pattern of **mature neutrophilia** (lack of a left shift), **lymphopenia**, and **eosinopenia** is consistent with **corticosteroid administration**. Neutrophilia results from increased bone marrow release of neutrophils as well as a shift in neutrophils from the marginal neutrophil pool (where they cannot be counted) to the circulating neutrophil pool (where they are enumerated by the WBC count). Lymphopenia and eosinopenia are due to cellular redistribution within the vasculature.

2. **Increased ALP activity with resistance to levamisole inhibition**. High ALP activity is induced by endogenous and/or exogenous corticosteroids. With sufficient exposure to corticosteroids, dogs produce a unique corticosteroid isoenzyme of ALP. The presence of this **corticosteroid ALP isoenzyme** is confirmed by the **high resistance of ALP to levamisole inhibition** (activities of both the hepatic and bone isoenzymes of ALP are inhibited by levamisole).

3. **Increased ALT activity** suggests some degree of hepatocellular enzyme leakage associated with corticosteroid hepatopathy. Hepatopathy is associated with glycogen loading and swelling of hepatocytes that may compress bile canaliculi and promote cholestasis. Cholestasis causes increased hepatocellular permeability because of the detergent-like effect of bile on plasma membranes.

4. **Hyperglycemia without glucosuria**. **Hyperglycemia** is attributed to gluconeogenesis from endogenous corticosteroid release and exogenous corticosteroid administration.

5. **Hyperadrenocorticism. Overstimulation with ACTH** and **failure to suppress plasma cortisol** in the low-dose dexamethasone test indicate hyperadrenocorticism but do not distinguish between pituitary-dependent and adrenal-dependent disease. A high-dose dexamethasone suppression test could be

www.Ebook777.com

helpful in making that distinction (most cases of pituitary-dependent disease suppress). The adrenal gland would fail to respond to ACTH stimulation with an increase in plasma cortisol concentration if the hyperadrenocorticism were iatrogenic due to the corticosteroid therapy.

6. Very low urine Sp Gr. The **very low urine Sp Gr** is probably the result of medullary washout and reduced medullary tonicity caused by the corticosteroid-induced polyuria. A gradual water deprivation test would be needed to completely exclude renal failure as a cause of polyuria.

SUMMARY: A pituitary adenoma was discovered at necropsy.

Free ebooks ==> www.Ebook777.com

CASE 27 HYPERTHYROIDISM

SIGNALMENT: Cat, domestic shorthair, female, 12 years old

PRESENTING PROBLEMS: Weakness, weight loss, polyphagia, tachycardia, dyspnea, pleural effusion, enlarged thyroid glands, fleas

LABORATORY DATA:

	Hematology		
Hct	48	H	%
Hb	16.6	H	g/dL
RBC	10.2	H	$\times 10^6/\mu L$
MCV	47.1		fL
MCH	16.3		pg
MCHC	34.6		%
RBC morphology: normal			
Platelets: adequate			
WBC	21.9	H	$\times 10^3/\mu L$
Seg	19.053 (87%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	0.876 (4%)	L	$\times 10^3/\mu L$
Mono	0.219 (1%)		$\times 10^3/\mu L$
Eos	1.752 (8%)	H	$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
	Urinalysis		
Urine source	voided		
Color	yellow		
Turbidity	clear		
Sp Gr	1.060		
pH	6.5		
Protein	negative		
Glucose	negative		
Ketone	negative		
Bilirubin	negative		
Blood	2+		
Sediment	30 to 50 RBC/hpf		
	0 to 1 WBC/hpf		
	fat droplets		

continued

www.Ebook777.com

	Serum Chemistry		
BUN	39	H	mg/dL
Creatinine	0.7		mg/dL
Total protein	7.2		g/dL
Albumin	3.6		g/dL
A/G ratio	1.0		
ALP	59	H	U/L
ALT	220	H	U/L
Glucose	132	H	mg/dL
Sodium	154		mmol/L
Potassium	3.9		mmol/L
Chloride	131		mmol/L
Total CO_2	13		mmol/L
Anion gap	13.9		mmol/L
Calcium	9.1		mg/dL
	Other Tests		
Tot thyroxine (TT_4)	23.4	H	µg/dL
Pleural fluid analysis:			
Volume	200		mL
Clear and colorless			
Protein	3.4		g/dL
Nucleated cell count	2,000		/µL
Small lymphocytes, a few nondegenerateneutrophils, macrophages, and mesothelial cells			
Opinion: modified transudate of cardiac disease			

PROBLEMS:

1. **Polycythemia.** The **increased hematocrit, hemoglobin, and RBC count** indicate **polycythemia**. Dehydration, a common cause of relative polycythemia, was not observed on the physical examination. Excitement may cause splenic contraction and relative polycythemia, but changes in the leukogram do not support physiologic leukocytosis from epinephrine release. Therefore, absolute polycythemia is probably present in this cat from hyperthyroidism with stimulation of erythropoiesis and, possibly, increased erythropoietin production.

2. **Leukocytosis, neutrophilia, lymphopenia,** and **eosinophilia**.

a. **Neutrophilic leukocytosis** and **lymphopenia** suggest stress with endogenous cortisol release.

b. **Eosinophilia.** Eosinophilia is frequently observed with hypersensitivity or parasitic infestations (including endo- and ectoparasites). Allergy and parasitism are not mutually exclusive as causes of eosinophilia. This cat may have flea allergy dematitis.

3. **Increased BUN concentration.** The mildly **increased BUN concentration** is probably the result of increased protein catabolism associated with hyperthyroidism. **Azotemia** and **high urine Sp Gr** (1.060) suggest prerenal azotemia, but dehydration is not apparent on physical examination. However, some azotemic cats may retain renal function sufficient to concentrate their urine during the progression of renal disease (but usually not to the degree observed in this cat). The creatinine concentration is within the reference interval despite an increased BUN concentration. This observation tends to exclude decreased GFR from dehydration.

Free ebooks ==> www.Ebook777.com

4. **Hepatic disease.**
   a. **Increased ALP activity.** ALP is an induction enzyme and generally serves as a marker of biliary disease. Hepatic centrilobular lesions usually do not cause cholestasis, so **increased ALP activity** must be explained by another mechanism. Hyperthyroidism is thought to cause increased bone turnover. Therefore, an increase in activity of the bone isoenzyme of ALP is a possibility. In humans, both hepatic and bone isoenzymes of ALP are increased during hyperthyroidism.
   b. **Increased ALT activity** suggests hepatocellular injury with leakage of this cytosolic enzyme. This change may represent centrilobular hepatocellular hypoxia secondary to hypertrophic cardiomyopathy.

5. **Hyperglycemia. Hyperglycemia** is observed occasionally in cats with **hyperthyroidism**. Possible explanations for the hyperglycemia include a catecholamine (epinephrine) response triggered by hyperexcitability. Alternatively, hyperglycemia may be due to gluconeogenesis incited by cachexia and muscle catabolism related to hyperthyroidism. In people with hyperthyroidism, hyperglycemia is associated with decreased insulin production and a resistance of peripheral tissues to insulin-facilitated glucose uptake.

6. **Hyperthyroidism.** A markedly increased serum thyroxine (T_4) concentration is diagnostic of hyperthyroidism. In cats, this disease usually is associated with thyroid nodular hyperplasia. Hypertrophic cardiomyopathy may develop during the progression of disease.

7. **Pleural effusion.** Secondary hypertrophic cardiomyopathy, resulting from hyperthyroidism, is the probable cause of the modified transudate. The three most common causes of lymphocyte-rich pleural effusions in cats are cardiac disease, chylothorax, and lymphoma.

SUMMARY: The cat was hyperthyroid and responded to radioactive iodine treatment.

www.Ebook777.com

CASE 28 VON WILLEBRAND'S DISEASE

SIGNALMENT: Canine, Doberman Pinscher, female, 2 years old

PRESENTING PROBLEMS: Continuous vaginal bleeding since parturition 8 weeks ago, skin rash that frequently bleeds

LABORATORY DATA:

	Hematology	
Hct	41	%
Platelets	253	$\times 10^3/\mu L$
WBC	8.3	$\times 10^3/\mu L$
	Urinalysis	
Urine source	voided	
Color	pink	
Turbidity	clear	
Sp Gr	1.036	
pH	6.5	
Chemistries	negative	
Blood	2+	
Sediment	50 to 60 RBC/hpf 0 to 1 WBC/hpf	
	Serum Chemistry	
BUN	17	mg/dL
Creatinine	0.7	mg/dL
Total protein	6.7	g/dL
Albumin	2.7	g/dL
A/G ratio	0.68	
ALP	51	U/L
ALT	75	U/L
Glucose	105	mg/dL
Sodium	146	mmol/L
Potassium	4.4	mmol/L
Chloride	117	mmol/L
Total CO_2	21	mmol/L
Anion gap	8	mmol/L
Calcium	10.3	mg/dL
Cholesterol	276	mg/dL

Free ebooks ==> www.Ebook777.com

	Other Tests		
BMBT	6	H	min
APTT	14.2		sec
PT	6.3		sec
FDP	<10		µg/mL
Fibrinogen	200		mg/dL
Von Willebrand's factor antigen	16	L	% of N

PROBLEMS:

1. **Von Willebrand's disease.** vWF deficiency causes platelet dysfunction and **prolonged buccal mucosal bleeding time (BMBT)**. Other causes of a prolonged BMBT when the platelet count is within the reference interval include uremia or increased FDPs which are not supported by the laboratory data. Coagulation factor VIII deficiency accompanies deficiency of vWF, but factor VIII activity is rarely <30% of the reference interval. Therefore, the APTT is generally not prolonged. vWD is confirmed by documenting decreased vWD factor antigen.

2. **Hematuria**. A **positive occult blood reagent strip test** and the presence of many **erythrocytes in the urine sediment** indicate hematuria. Blood in a voided urine sample could be of genital tract origin, which is a possibility in this dog.

SUMMARY: An ovariohysterectomy was performed successfully. Fresh or frozen plasma should be available and administered, if needed, to control any bleeding tendency associated with vWD. Platelets are not needed in the transfusion because the defect is a lack of plasma glycoprotein (vWF); the platelets are normal.

www.Ebook777.com

CASE 29 UREA TOXICOSIS

SIGNALMENT: Bovine, Holstein, cow (female), 7 months old

MEDICAL HISTORY: One of a group of cattle accidentally fed a diet containing toxic levels of urea 72 hours prior to admission. The heifer has been sternally recumbent for at least 2 days.

PRESENTING PROBLEMS: Large, splashy, atonic rumen; arrhythmia suggestive of atrial fibrillation; diffuse fine muscular tremors of face, neck, and flank

LABORATORY DATA:

	Hematology		
Hct	37.1	L	%
Hb	13.2	L	g/dL
RBC	10.67	L	$\times 10^6/\mu L$
MCV	34.8		fL
MCH	12.4		pg
MCHC	35.6		%
RBC morphology: slight anisocytosis			
Platelets:	720		$\times 10^3/\mu L$
WBC	44.6	H	$\times 10^3/\mu L$
Seg	36.126 (81%)	H	$\times 10^3/\mu L$
Lymph	6.244 (14%)		$\times 10^3/\mu L$
Mono	2.230 (5%)	H	$\times 10^3/\mu L$
Eos	0.0		$\times 10^3/\mu L$
Baso	0.0		$\times 10^3/\mu L$
WBC morphology: normal			
Plasma color pale yellow, clear			
Plas prot	8.1	H	g/dL
Fibrinogen	700	H	mg/dL
	Urinalysis		
Urine source	voided		
Color	amber		
Turbidity	cloudy		
Sp Gr	1.029		
pH	8.0		
Protein	negative		
Glucose	1+		
Ketone	negative		

Free ebooks ==> www.Ebook777.com

Bilirubin	negative		
Blood	negative		
Sediment	0 RBC/hpf 0 to 2 squamous cells/hpf many triple phosphate		
	Serum Chemistry		
BUN	44	H	mg/dL
Creatinine	1.1		mg/dL
Total protein	7.9	H	g/dL
Albumin	3.2		g/dL
Globulin 4.7 H g/dL			
A/G ratio	0.68		
GGT	19	H	U/L
AST	243	H	U/L
SDH	30	H	U/L
CPK	23,163	H	U/L
ALT	151	H	U/L
Glucose	87		mg/dL
Sodium	167	H	mmol/L
Potassium	3.9		mmol/L
Chloride	115	H	mmol/L
Total CO_2	34		mmol/L
Anion gap	22	H	mmol/L
Calcium	9.2		mg/dL
Phosphorus	7.8		mg/dL
Total bilirubin	1.7	H	mg/dL
	Other Tests		
Plasma NH_3	148	H	µg/dL
Rumen NH_3	38,935	H	µg/dL

PROBLEMS:

1. **Polycythemia. Increased hematocrit, hemoglobin concentration, and RBC count** indicate **polycythemia**. Mild hemoconcentration may be secondary to dehydration, but another physical examination will be necessary to confirm this impression. The hematocrit, MCV, and serum protein concentration often are slightly low in cattle of this age. Once the primary problem has been corrected and the heifer has been rehydrated, these parameters may drop to the low end of the reference interval.

2. **Anisocytosis**. Anisocytosis is a variation in erythrocyte size and is a normal finding in cattle.

3. **Leukocytosis, neutrophilia, and monocytosis**.
   a. **Leukocytosis** and marked **neutrophilia**. Neutrophilia may be due to excitement and catecholamine response associated with shipping as well as inflammation. Neutrophilia from excitement is transient, whereas neutrophilia from inflammation is more persistent. **Hyperfibrinogenemia** suggests that inflammation is present. Possible sites of inflammation are the rumen wall and the skeletal muscles.
   b. **Monocytosis**. **Monocytosis** can occur in acute or chronic disease. Blood monocytes provide a replacement pool for tissue macrophages.

4. **Hyperproteinemia (plasma) and hyperfibrinogenemia**. A portion of the **hyperproteinemia** in **plasma** is due to **hyperfibrinogenemia**. This finding indicates inflammation.

www.Ebook777.com

5. High anion gap. The elevated anion gap may be due to lactic acid production from shock; however, the TCO_2 is within the reference interval. Blood gas analysis would give a more detailed picture of acid-base alterations.

6. Hypernatremic hypovolemia. The presence of a large amount of a small molecular weight substance (urea) has increased the tonicity of rumen fluid. Little urea diffuses out of the rumen, but water can diffuse in. Water has transferred from the ECF to the rumen, but Na^+ has remained in the ECF, resulting in **hypernatremia** and **hypovolemia**. The total body water is normal, but there is dehydration of the ECF and ICF, and accumulation of water in the GI tract.

7. Hyperosmolality of ECF. The ECF is hyperosmolal: (167 + 3.9) (2) = 341.8 mOsm/L. The ECF hypernatremia has drawn water from the ICF, partially masking dehydration as assessed by signs of hypovolemia. Loss of ICF water leads to cell shrinkage, possibly contributing to the neurologic abnormalities. Determination of serum osmolality and calculation of the osmolal gap would help determine whether unmeasured, nonpolar compounds have been absorbed from the rumen. Rumen fluid osmolality would help determine the severity of the osmolar gradient between it and the ECF.

8. Possible potassium depletion. Although the serum K^+ is normal, it is possible that total body K^+ deficit exists. Potassium may be leaking from damaged muscles, increasing ECF K^+. However, the heifer has been anorectic, decreasing ECF K^+. The cardiac arrhythmia may be an indication of ICF K^+ depletion. Sequential serum K^+ monitoring is warranted.

9. Hyperchloridemia. Most of the **hyperchloridemia** is due to increased NaCl. The Na-Cl difference (52) is increased slightly (reference interval 45 to 53), indicating a selective disorder in Cl^- is present. The slight increase in Cl^- (compared to the marked increase in Na^+) is most likely due to abomasal stasis and sequestration of HCl secondary to the metabolic disorder.

10. Increased BUN concentration. Some of the urea may have been absorbed from the lower GI tract. Another possibility is increased hepatic synthesis of urea from ammonia as the body tries to clear the excess ammonia. If dehydration is present, prerenal azotemia also is a consideration.

11. Hyperproteinemia, hyperglobulinemia, and **A/G ratio within the reference interval**. The **hyperproteinemia** is characterized by a proportional increase in albumin and globulin due to hypovolemia (loss of plasma water).

12. Increased AST activity. AST activity is high in many cells including hepatocytes, skeletal muscle cells, and erythrocytes. The SDH is within the reference interval and hemolysis is not present in the plasma or serum, indicating that increased serum enzyme activity is from muscle injury (see below).

13. Markedly increased CK activity. Increased CK activity in unhemolyzed serum is indicative of muscle injury. Pressure damage of skeletal muscles may occur during prolonged recumbency in cattle (downer cow). Another possible cause of muscle damage is myocyte shrinkage from hypernatremia.

14. Increased ALT activity. The cytosol of hepatocytes contains little ALT activity in large animals. The serum activity of this enzyme is seldom measured in these species. Elevated serum ALT activity in cattle may be associated with muscle damage.

15. Increased GGT activity. ALP has a very broad reference interval in large animals and is not clinically useful as a marker of biliary disease. GGT activity is a more sensitive indicator of biliary disease in large animals. The increased serum GGT activity suggests that mild biliary disease is present. In this cow, GGT activity probably has been induced via cholestasis.

16. Increased SDH activity. **SDH** is an indicator of hepatocellular damage. It is located in the cytoplasm of hepatocytes and leaks into the blood when hepatocellular membrane integrity is compromised.

Free ebooks ==> www.Ebook777.com

17. **Hyperammonemia**. Rumen microflora convert urea to ammonia, which can be absorbed into the blood in the form of NH_3. Ammonia (and possibly other amines) contribute to neurologic abnormalities and muscle weakness.

18. **Normoglycemia and mild glucosuria**. Transient hyperglycemia may produce glucosuria if blood glucose exceeds the renal threshold. Hyperglycemia may be transient (e.g., excitement and catecholamine release) but urine is stored in the bladder for a variable time. Therefore, glucosuria will be accompanied by normoglycemia when excitement is no longer apparent. Another cause of normoglycemia and glucosuria could be acute renal disease with renal tubular necrosis, but the urinalysis findings do not support this possibility.

19. **Mildly increased total bilirubin concentration**. Anorectic cattle often have slight **hyperbilirubinemia**.

20. **Alkaline urine with triple phosphate crystalluria**. An anorectic animal usually excretes acid urine. The urine is alkaline because of the excretion of excess ammonia. Triple phosphate crystals form in alkaline urine, leading to their presence in the sediment.

SUMMARY: Urea toxicosis resulted in fluid shifts into the rumen, causing hypernatremic dehydration. The rumen flora converted the urea to ammonia, some which was absorbed, leading to hyperammonemia and ammonia toxicity. Consequences of ammonia toxicity include disorientation (and other neurologic deficits) and muscle weakness. Subsequent prolonged recumbency caused further muscle damage and limited access to food and water. Excretion of excess ammonia via the kidneys produced alkaline urine and triple phosphate crystalluria. The heifer was treated with IV fluids containing isotonic NaCl and 5% glucose to slowly lower the serum Na^+ without producing secondary cerebral swelling. Two gallons of rumen fluid from a healthy cow was given to re-establish normal rumen flora. Within 6 hours, the heifer was improving and passing foul smelling feces. She eventually recovered after 6 days of supportive care.

www.Ebook777.com

CASE 30 END STAGE RENAL DISEASE WITH UREMIC PNEUMONITIS

SIGNALMENT: Canine, Bull Mastiff, male, 2 years old

MEDICAL HISTORY: Polyuria, diagnosed with congenital renal disease at 2 months of age, deterioration despite supportive care, dialysis, and transfusion 2 weeks ago

PRESENTING PROBLEMS: Severe respiratory distress, oliguria

RADIOGRAPHIC FINDINGS: Moderate pulmonary edema and mineralization of the tracheal rings

LABORATORY DATA:

	Hematology		
Hct	35.7		%
Hb	12.7		g/dL
RBC	5.19		$\times 10^6/\mu L$
MCV	68.8		fL
MCH	24.5		pg
MCHC	35.5		%
RBC morphology: normal			
Platelets:	131	L	$\times 10^3/\mu L$
WBC	20.8	H	$\times 10^3/\mu L$
Seg	19.74 (95%)	H	$\times 10^3/\mu L$
Band	0.0		$\times 10^3/\mu L$
Lymph	0.330 (2%)	L	$\times 10^3/\mu L$
Mono	0.530 (3%)		$\times 10^3/\mu L$
Eos	0.000 (0%)		$\times 10^3/\mu L$
Baso	0.000 (0%)		$\times 10^3/\mu L$
WBC morphology: normal			
Plasma color: colorless, clear			
Plas prot	7.4		g/dL
	Urinalysis		
Urine source	cystocentesis		
Color	yellow		
Turbidity	clear		
Sp Gr	1.010		
pH	5.0		

Free ebooks ==> www.Ebook777.com

Protein	1+		
Glucose	negative		
Ketone	negative		
Bilirubin	negative		
Blood	negative		
Sediment	0 RBC/hpf		
	0 to 2 WBC/hpf		
	0 to 2 transitional/hpf		
	amorphous crystal		
	Arterial blood gas analysis (on room air)		
pH	7.144	L	
pCO_2	43.2	H	mmHg
pO_2	65.5	L	mmHg
HCO_3^-	13.9	L	mmol/L
BE	−13.1	L	mmol/L
	Serum Chemistry		
BUN	41	H	mg/dL
Creatinine	10.8	H	mg/dL
Total protein	5.0	L	g/dL
Albumin	2.2	L	g/dL
Globulin	3.8		g/dL
A/G ratio	0.58	L	
Glucose	45	L	mg/dL
Sodium	148		mmol/L
Potassium	5.5	H	mmol/L
Chloride	110		mmol/L
Total CO_2	13	L	mmol/L
Anion gap	29	H	mmol/L
Calcium	13.9	H	mg/dL
Phosphorus	16.2	H	mg/dL

PROBLEMS:

1. **Leukocytosis, neutrophilia**, and **lymphopenia**. Stress with endogenous cortisol release has resulted in these leukogram changes. Neutrophilia is the result of increased release of neutrophils from the bone marrow into the blood as well as decreased emigration of neutrophils from the blood into the tissues. Lymphopenia is due to temporary redistribution of recirculating lymphocytes.

2. **Hypoventilatory respiratory disease**. Hypoxia with **increased PCO_2** (**respiratory acidosis**) is occurring. The combination of **low PO_2** (**hypoxia**) and increased PCO_2 (**hypercapnia**) indicate decreased pulmonary ventilation. The effective flow of air in and out of alveoli has been impeded. Depression of the respiratory centers is excluded because of the observed respiratory effort. Both pneumothorax and hydrothorax have been excluded by radiography. Therefore, some form of obstructive respiratory disease is present. Upper airway obstruction is excluded by physical examination, leaving **intrapulmonary obstructive disease** as the cause of these laboratory abnormalities.

3. **Mixed metabolic and respiratory acidosis**. The expected metabolic compensation for **respiratory acidosis** is to increase HCO_3 concentration by decreased excretion through the kidneys. However, this

www.Ebook777.com

dog also has a **metabolic acidosis** from prolonged renal disease. The combination of **two forms of acidosis** has led to a profound decrease in blood pH (**acidemia**).

4. **High anion gap with metabolic acidosis**. The **increased anion gap** indicates the presence of unmeasured anions. The **decreased HCO_3 concentration** indicates **metabolic acidosis**. The cause of the acidosis is titration with acids, including phosphoric and uremic acids. More precise information on the patient's acid- base status is obtained via blood gas analysis.

5. **Hyperkalemia**. The dog is in the terminal phase of oliguric renal disease, converting from a polyuric renal disease to **oliguric renal disease**. Consequently, K^+ is retained, leading to **hyperkalemia**. The acidosis probably is the cause of increased K^+, because the forms of acidosis present (titration and respiratory acidosis) tend not to produce ICF to ECF K^+ shifts.

6. **Hypercalcemia, hyperphosphatemia,** and **increased Ca × P product**. The combination of increased calcium and phosphorus concentrations may be seen in **congenital renal disease**. The **Ca × P product = 150.1**. Therefore, the dog is at **high risk for mineralization of soft tissue**.

7. **Azotemia**. Markedly **increased creatinine** and mildly **increased BUN** concentrations are present. The previous dialysis has reduced BUN concentration by increasing the rate of urine production, but has not decreased the creatinine concentration.

8. **Hypoglycemia**. The dog has probably not eaten and is using glucose in the exertion of labored breathing. **Hypoglycemia** may reflect the **exhaustion of glycogen stores**.

9. **Hypoproteinemia, hypoalbuminemia, and decreased A/G ratio**. The calculated **A/G ratio** is decreased (**0.58**), whereas globulin concentration is within the reference interval. Therefore, **hypoproteinemia** is caused by **hypoalbuminemia**. Selective loss of albumin in the urine is caused by congenital renal disease. A 1+ proteinuria suggests marked protein loss in dilute urine.

10. **Isosthenuria and mild proteinuria**. A fixed urine specific gravity and proteinuria are consistent with end stage renal disease. Urine reagent strips are more sensitive in detecting albumin.

SUMMARY: The dog did not respond to oxygen therapy, and was euthanatized. Following necropsy and histopathology, diffuse mineralization of alveolar walls was found throughout the lungs (uremic pneumonitis). This mineralization prevented normal elastic recoil of alveoli, leading to inability to expire air and obstructive, hypoventilatory pulmonary disease. The kidneys were markedly small and fibrotic (end stage renal disease).

Free ebooks ==> www.Ebook777.com

CASE 31 EARLY PRIMARY HYPOTHYROIDISM

SIGNALMENT: Canine, English Springer Spaniel, male, 2 years old

MEDICAL HISTORY: Hypothyroidism has been documented in this dog's lineage

PRESENTING PROBLEMS: Dog is asymptomatic but owner desires testing for hypothyroidism

PHYSICAL FINDINGS: Corneal cholesterol deposits

ENDOCRINE LABORATORY DATA:[a]

Test	*Patient Value*	*Reference Interval*[b]
Total T4	0.78	(1.5 to 4.0 µg/dL)
Total T3	104	(78 to 260 ng/dL)
Free T4 (by dialysis)	0.7	(0.9 to 2.3 ng/dL)
Anti-thyroglobulin autoantibodies	negative	negative
Anti-T4 autoantibodies	6.0 %	(<10 %)
Anti-T3 autoantibodies	0.0%	(<2 %)
cTSH	1.2 ng/ml	(<0.6 ng/mL)

1. **Reference intervals.** Each laboratory performing endocrine testing should establish their own species-specific reference intervals. Commercial assay kits intended for use with human specimens must be modified for use with animal specimens.

2. **Decreased total T4, low free T4** (by dialysis), and **increased cTSH** concentrations are consistent with **early primary hypothyroidism**. Total T3 concentration may remain within the reference interval as peripheral tissues and the thyroid gland produce a larger percentage of T3. This dog does not have obvious evidence of autoimmune thyroiditis because anti-thyroglobulin, anti-T4, and anti-T3 autoantibody concentrations are within the reference interval.

COMMENT: This dog has early hypothyroidism. It would be reasonable to treat this patient with thyroid replacement therapy (L-thyroxine). This dog should not be bred because of the history of hypothyroidism in its lineage.

[a] A complete blood cell count and biochemical profile are recommended in the diagnostic evaluation of patients with presumed or suspected endocrine disease. In this patient, hematologic and biochemical abnormalities were minimal to absent and have been omitted to simplify discussion of the endocrine data.

[b] The reader is referred to the official website of the Society for Comparative Endocrinology (http://www.compendo.org) for a list of laboratories that perform validated endocrine diagnostic testing and furnish associated reference intervals.

www.Ebook777.com

CASE 32 POSSIBLE GLUCOCORTICOID SUPPRESSION OF TYHROID FUNCTION

SIGNALMENT: Canine, Golden Retriever, castrated male, 8 years old

MEDICAL HISTORY: Treatment with 20 mg prednisolone given orally every 48 hours for the past 18 months to control pruritus

PRESENTING PROBLEMS: Obesity, slightly dry hair coat

PHYSICAL FINDINGS: Obesity (body weight = 107 pounds)

ENDOCRINE LABORATORY DATA:[a]

Test	*Patient Value*	*Reference Interval*[b]
Total T4	<0.5	(1.5 to 4.0 µg/dL)
cTSH	0.2	(<0.6 ng/mL)

1. **Reference intervals.** Each laboratory performing endocrine testing should establish their own species-specific reference intervals. Commercial assay kits intended for use with human specimens must be modified for use with animal specimens.

2. **Decreased total T4 and TSH concentrations**. A **decreased total T4 concentration** is present and is probably related to treatment with an anti-inflammatory dosage of prednisolone for the past 1 1/2 years. **Suppression of TSH concentration** by **glucocorticoid administration** has been difficult to document, although it is presumed to be one of the mechanisms associated with the low total T4 concentration. Glucocorticoid-associated suppression of TSH concentration into the reference interval has not been demonstrated, even in dogs with experimentally-induced hypothyroidism. However, **approximately 25% of dogs with hypothyroidism may have a TSH value that is within the reference interval**. If further evaluation of the thyroid axis is needed, the patient should be weaned from prednisolone over a period of approximately 1 month. The patient's thyroid axis should be retested when complete prednisolone withdrawal has been in effect for at least 6 weeks.

[a]A complete blood cell count and biochemical profile are recommended in the diagnostic evaluation of patients with presumed or suspected endocrine disease. In this patient, hematologic and biochemical abnormalities were minimal to absent and have been omitted to simplify discussion of the endocrine data.

[b]The reader is referred to the official website of the Society for Comparative Endocrinology (http://www.compendo.org) for a list of laboratories that perform validated endocrine diagnostic testing and furnish associated reference intervals.

Free ebooks ==> www.Ebook777.com

CASE 33 PRIMARY HYPOTHYROIDISM (AND PHENOBARBITAL ADMINISTRATION)

SIGNALMENT: Canine, Miniature Poodle, male, 5 years old

MEDICAL HISTORY: Persistent lipemia, daily treatment with phenobarbital for epilepsy

PRESENTING PROBLEMS: Lethargy, hair loss

PHYSICAL FINDINGS: Nonpruritic, bilaterally symmetrical alopecia

ENDOCRINE LABORATORY DATA:[a]

Test	*Patient Value*	*Reference Interval*[b]
FT4 (by dialysis)	0.4	(0.9 to 2.3 ng/dL)
cTSH	3.2	(<0.6 ng/mL)

1. **Reference intervals.** Each laboratory performing endocrine testing should establish their own species-specific reference intervals. Commercial assay kits intended for use with human specimens must be modified for use with animal specimens.

2. **Decreased free T4 and increased TSH concentrations.** Decreased total T4 and free T4 are known to occur with phenobarbital treatment, but these changes are mild and not necessarily associated with the dramatically increased TSH concentration observed in this dog. These laboratory test results are consistent with primary hypothyroidism. Thyroid replacement therapy with L-thyroxin should be instituted.

[a]A complete blood cell count and biochemical profile are recommended in the diagnostic evaluation of patients with presumed or suspected endocrine disease. In this patient, hematologic and biochemical abnormalities were minimal to absent and have been omitted to simplify discussion of the endocrine data.

[b]The reader is referred to the official website of the Society for Comparative Endocrinology (http://www.compendo.org) for a list of laboratories that perform validated endocrine diagnostic testing and furnish associated reference intervals.

www.Ebook777.com

CASE 34 HYPERADRENOCORTICISM AND DIABETES MELLITUS WITH KETOACIDOSIS, HYPEROSMOLALITY, NECROTIZING PANCREATITIS, AND URINARY TRACT INFECTION

SIGNALMENT: Canine, Miniature Poodle, male, 5 years old

PRESENTING PROBLEMS: Depression, weakness, vomiting, diarrhea

LABORATORY DATA:

	Hematology		
Hct	43		%
Hb	15.3		g/dL
RBC	5.1		$\times 10^6/\mu L$
MCV	85	H	fL
MCH	30	H	pg
MCHC	35.6		%
RBC morphology: occasional macrocytes			
Platelets:	350		$\times 10^3/\mu L$
WBC	39	H	$\times 10^3/\mu L$
Seg	35.490 (91%)	H	$\times 10^3/\mu L$
Band	0.780 (2%)	H	$\times 10^3/\mu L$
Lymph	0.780 (3%)		$\times 10^3/\mu L$
Mono	1.950 (5%)	H	$\times 10^3/\mu L$
Eos	0.000 (0%)		$\times 10^3/\mu L$
Baso	0.000 (0%)		$\times 10^3/\mu L$
WBC morphology: cytoplasmic basophilia and vacuolation			
Modified Knott's test: *Dirofilaria immitis* present			
	Urinalysis		
Urine source	catheterized		
Color	dark yellow		
Turbidity	cloudy		

Free ebooks ==> www.Ebook777.com

Sp Gr	1.039		
pH	8.0		
Protein	2+		
Glucose	2+		
Ketone	1+		
Bilirubin	negative		
Blood	trace		
Sediment	10 to 15 RBC/hpf 8 to 10 WBC/hpf 1 to 4 granular casts/hpf bacilli, spermatozoa, fat droplets		
	Arterial blood gas analysis (on room air)		
pH	7.282	L	
pCO_2	28.3	H	mmHg
pO_2	85.1		mmHg
HCO_3^-	12.9	L	mmol/L
	Serum Chemistry		
BUN	53	H	mg/dL
Creatinine	2.9	H	mg/dL
Total protein	9.7	H	g/dL
Albumin	3.7	H	g/dL
Globulin	4.2		g/dL
A/G ratio	0.88		
ALT	331	H	U/L
ALP	1,321	H	U/L
ALP w/ levam	998		U/L
Glucose	160	H	mg/dL
Sodium	159	H	mmol/L
Potassium	4.2		mmol/L
Chloride	87		mmol/L
Total CO_2	13	L	mmol/L
Anion gap	29	H	mmol/L
Calcium	9.8		mg/dL
Phosphorus	16.2	H	mg/dL
Total bilirubin	0.3		mg/dL
Amylase	2,422	H	U/L
Lipase	744	H	U/L
	Other Tests		
Baseline cortisol	9.8	H	µg/dL
Low dose dexameth	7.2	H	µg/dL
High dose dexameth	5.5	H	µg/dL

PROBLEMS:

1. **Macrocytosis.** The mean corpuscular volume is increased, indicating **macrocytosis**. A few macrocytes are noted on the stained blood smear, but **polychromasia is not present**. The red blood cell count is near the lower limit of the reference interval. These laboratory findings are consistent with **congenital macrocytosis** that is observed occasionally in Poodles.

www.Ebook777.com

2. Increased MCH but MCHC is within the reference interval. The **MCH** and **MCHC** usually indicate similar trends in hemoglobin content within erythrocytes. When these values disagree, the interpretation should be based upon the MCHC. Of the three erythrocytic indices (MCV, MCH, and MCHC), the MCHC is more accurate because it does not require an RBC count for its mathematical calculation. Thus, the erythrocyte population is **normochromic**.

3. Leukocytosis, neutrophilia with toxic change, and monocytosis.

a. **Leukocytosis** is primarily due to a mature neutrophilia.

b. **Neutrophilia** suggests an increased tissue demand for phagocytes and/or a response to corticosteroids. A clinically important left shift (>1,000 bands/μL) is not yet present but should be monitored by sequential leukograms.

c. **Toxic change of neutrophils** suggests **infection or severe inflammation**; urinary tract infection is present in this patient.

d. The **lymphocyte count is depressed** but still within the reference interval; a downward trend in the lymphocyte count could be confirmed by sequential leukograms. The depressed lymphocyte count may be due to temporary redistribution of recirculating lymphocytes from endogenous cortisol release.

e. **Monocytosis** is present and may accompany **acute or chronic disease**. Neutrophilia and monocytosis in the dog also may reflect a leukocyte **response to exogenous or endogenous corticosteroids**.

4. Heartworm infestation. The modified Knott's test reveals microfilaremia due to *Dirofilaria immitis* infection (heartworm disease). Eosinophilia and basophilia often accompany heartworm disease. The lack of eosinophilia and basophilia suggests that the patient may be releasing excess endogenous cortisol from stress or hyperadrenocorticism.

5. Urinary tract infection, hematuria, possible proteinuria, glucosuria, and ketonuria.

a. **Pyuria** and **bacteriuria** in a urine specimen obtained by catheter indicates urinary tract infection.

b. Urinary pH is expected to be acidic in carnivores. **Alkaluria** suggests that the **bacteria are degrading urea to ammonia**.

c. The presence of **erythrocytes** and a **positive dipstick test for blood** indicate **mild hematuria**.

d. The **protein reagent pad** will change color nonspecifically in **alkaline urine**; therefore, the presence of proteinuria must be confirmed by another technique such as acid precipitation (**Robert's test**).

e. **Glucosuria with concurrent hyperglycemia** indicates **diabetes mellitus**.

f. **Ketonuria** suggests a **negative energy balance.** Ketonuria is detected specifically by the **nitroprusside reaction** via dipstick analysis and explains the presence of metabolic acidosis with an increased anion gap in the biochemical profile.

6. Uncompensated metabolic acidosis with hypernatremia, hypochloridemia, and an increased anion gap. Metabolic acidosis is present in conjunction with an **increased anion gap**, suggesting a titration-type acidosis. The increased anion gap indicates that the **unmeasured anions** could be ketone bodies or lactate from anaerobic metabolism secondary to shock. The urinalysis, however, confirms the presence of ketones. In this patient, **ketoacids** probably are titrating plasma bicarbonate. **Hypocapnia** is equivocal but may indicate that the depth and/or rate of ventilation have been increased to promote CO_2 release, but the acidosis is still uncompensated. Excessive chloride is being lost via vomiting resulting in hypernatremia.

7. Azotemia. The azotemia is probably prerenal in origin. Dehydration commonly accompanies vomiting and diarrhea and will result in a decreased glomerular filtration rate. Post-renal azotemia from urethral obstruction is excluded because the urinary catheter was passed easily. Renal azotemia is excluded because the kidneys are concentrating the urine to conserve water in the presence of putative dehydration.

Free ebooks ==> www.Ebook777.com

8. Hyperproteinemia, hyperalbuminemia, and A/G ratio within the reference interval. The A/G ratio indicates that both albumin and globulin are increased proportionally due to dehydration. The body does not over-synthesize albumin.

9. Increased ALT activity. Increased ALT activity indicates **hepatocellular leakage**. In this patient, hepatocellular leakage could be due to cholestasis with a detergent action of bile on cell membranes and/or injury of hepatocytes from pancreatic enzyme release into the peritoneal cavity. Steroid hepatopathy also may contribute to ALT leakage, presumably from cholestasis-associated membrane damage and/or hepatocellular swelling from excessive glycogen accumulation (steroid hepatopathy).

10. Increased ALP activity with resistance to levamisole inhibition. Increased ALP activity occurs from **enzyme induction**. In this patient, much of the enzyme activity is **resistant to levamisole inhibition** and indicates the presence of the **steroid isoenzyme of ALP**, which is unique in dogs. **Cholestasis** may have originated from **regional inflammation associated with pancreatitis** and/or **compression of bile canaliculi with hepatocellular swelling. Cholestasis** induces increased activity of the **hepatic isoenzyme of ALP**.

11. Hyperglycemia with glucosuria. Hyperglycemia with concurrent **glucosuria** indicates **diabetes mellitus**.

12. Hyperosmolality. Serum osmolality can be estimated using the formula: mOsmol/kg = 2 [Na^+ + K^+ (mmol/L)] + [glucose (mg/dl) ÷ 18] + [urea (mg/dl) ÷ 2.8]) = mOsmol/kg. The estimated serum osmolality in this patient is: (167.4) + (8.9) + (2.9) = 354.2 mOsmol/kg.

13. Hyperphosphatemia with an increased Ca × P product. Hyperphosphatemia is probably secondary to the decreased elimination of phosphorus because of a decreased glomerular filtration rate. The calcium × phosphorus product is high (approximately 159), suggesting that mineralization of soft tissue is eminent.

14. Hyperamylasemia and hyperlipasemia. Hyperamylasemia and hyperlipasemia are suggestive of acute pancreatitis, but this should be confirmed by ultrasonography. With decreased GFR, the plasma clearance half life of these enzymes may be prolonged.

15. Increased baseline cortisol concentration with failure to suppress following low dose and high dose dexamethasone suppression tests. A high baseline cortisol concentration with a failure to suppress the plasma cortisol concentration with low and high doses of dexamethasone suggests a functional adrenocortical neoplasm.

SUMMARY: This dog had hyperadrenocorticism and diabetes mellitus that were complicated by ketoacidosis, hyperosmolality, acute necrotizing pancreatitis, and urinary tract infection. Ketoacidosis, hyperosmolality, and diabetes mellitus initially were managed with fluid therapy and insulin administration. Necrotizing pancreatitis ultimately resulted in persistent diabetes mellitus and exocrine pancreatic insufficiency that required daily treatment with insulin and oral pancreatic extracts. The urinary tract infection was eliminated with antibiotic therapy. An adrenocortical neoplasm was suspected based on the plasma cortisol data. A unilateral adrenal mass was observed by ultrasonography and adrenalectomy was performed. The mass was diagnosed histologically as an adrenocortical adenoma. Following surgery, the plasma cortisol concentration returned to the reference interval.

www.Ebook777.com

CASE 35 ARTIFACTS OF LIPEMIA, HEMOLYSIS, AND IMPROPER SAMPLE HANDLING

SIGNALMENT: Canine, Akita, female, 9 years old

PRESENTING PROBLEMS: Seizures, depression, vomiting, diarrhea

COMMENT: Specimen mailed to laboratory, biochemical profile specimen received on clot

LABORATORY DATA:

	Hematology		
Hct	45		%
Hb	19.6	H	g/dL
RBC	6.61		$\times 10^6/\mu L$
MCV	68.1		fL
MCH	29.7	H	pg
MCHC	43.6	H	%
RBC morphology: normal			
Plasma color: 4+ lipemia, 4+ hemolysis			
Platelet estimate: adequate			
Platelets:	833	H	$\times 10^3/L$
WBC	20.9	H	$\times 10^3/\mu L$
Seg	15.466 (74%)	H	$\times 10^3/\mu L$
Band	3.334 (16%)	H	$\times 10^3/\mu L$
Lymph	0.836 (3%)		$\times 10^3/\mu L$
Mono	1.045 (5%)		$\times 10^3/\mu L$
Eos	0.200 (1%)		$\times 10^3/\mu L$
Baso	0.000 (0%)		$\times 10^3/\mu L$
WBC morphology: cells look old, differential may be inaccurate, slight toxic basophilia			
	Serum Chemistry		
BUN	30	H	mg/dL
Creatinine	1.9	H	mg/dL
Total protein	7.9	H	g/dL

Free ebooks ==> www.Ebook777.com

Albumin	3.2	H	g/dL
Globulin	4.7	H	g/dL
A/G ratio	0.68		
ALT	198	H	U/L
ALP	112		U/L
Glucose	31	L	mg/dL
Sodium	145		mmol/L
Potassium	5.9	H	mmol/L
Chloride	112		mmol/L
Total CO_2	11	L	mmol/L
Anion gap	28	H	mmol/L
Calcium	11.9	H	mg/dL
Phosphorus	2.0	L	mg/dL
CK	422	H	U/L
Amylase	1,099	H	U/L
Lipase	55	L	U/L

PROBLEMS:

1. **Increased hemoglobin, MCH, and MCHC.** Because erythrocytes do not produce excessive quantities of hemoglobin, the presence of **hyperchromia indicates hemolysis**. This is verified by the observation of 4+ hemolysis in the plasma for the complete blood cell count (CBC) and in the serum submitted for the biochemical profile. If the hemoglobin value is falsely elevated, then the calculated MCH and MCHC will be erroneous. Hemolysis in laboratory specimens is observed most commonly as an *in vitro* artifact. The presence of lipemia also promotes hemolysis.

2. **Possible pseudothrombocytosis.** The platelet count is increased, but the platelet estimate from the stained blood smear appears adequate. Fragments from degenerating leukocytes could be counted incorrectly as platelets by the hematology analyzer.

3. **Leukocytosis, neutrophilia, left shift, toxic change, and degenerative leukocytes.**
   a. The leukocyte differential count may be erroneous because the leukocytes are degenerate and difficult to identify precisely. In some instances, cells are so old that a differential count cannot be done at all.
   b. The presence of a left shift suggests severe inflammation or infection with a tissue demand for phagocytes.
   c. The slight cytoplasmic basophilia could indicate real toxic change or a nonspecific change in cytoplasmic tinctorial property from prolonged exposure to EDTA anticoagulant.
   d. If a delay is anticipated in shipping the CBC count specimen to the laboratory, 2 air-dried blood smears should be made to reflect leukocyte morphology at the time the sample was procured. The EDTA tube can be refrigerated and will produce acceptable leukocyte and platelet counts for approximately 24 hours post collection.

4. **Mild azotemia**. The azotemia could be prerenal (from vomiting and diarrhea), renal, or post-renal in origin. A urine specimen was not submitted but is needed to distinguish the cause of the azotemia.

5. **Hyperproteinemia, hyperalbuminemia, hyperglobilinemia, and A/G ratio within the reference interval.** The protein values are difficult to interpret with certainty. Severe hemolysis will increase the total protein concentration because of the presence of hemoglobin. Lipemia may decrease light transmittance through the serum specimen in the spectrophotometer, resulting in false high values, especially in colorimetric endpoint assays. Such tests are commonly used to quantify total protein and

www.Ebook777.com

albumin. The possibility of dehydration also is a consideration for the elevated protein values and requires further investigation.

6. Increased ALT activity. The mild increase in ALT activity may be the result of increased hepatocellular leakage or intracellular release of ALT associated with hemolysis of erythrocytes (increased serum activity of AST and LDH also accompany hemolysis).

7. Hypoglycemia (possible pseudohypoglycemia). The serum specimen for biochemical testing was submitted on the clot. At room temperature, erythrocytes continue to consume glucose via anaerobic metabolism. The glucose concentration may decrease up to 10%/hour. It cannot be determined if the seizure activity in this patient resulted from hypoglycemia or if pseudohypoglycemia is an artifact of erythrocyte metabolism of glucose.

8. Hyperkalemia (possibly pseudohyperkalemia). Hyperkalemia may accompany oliguria, anuria, acidosis, or hemolysis in certain animal species. Certain Asian breeds of dogs (e.g., Akita, Shiba Inu), horses, and pigs have high intraerythrocytic potassium concentrations. Overt or covert hemolysis of erythrocytes may result in fictitious hyperkalemia. Markedly increased platelet counts also can cause an increase in serum K^+ concentration; however, pseudothrombocytosis is probably present in this dog (see above).

9. Decreased TCO_2 with an increased anion gap. The **decreased TCO_2** suggests **metabolic acidosis**; however, the serum specimen was submitted on the clot. As erythrocytes continue to metabolize glucose, lactic acid is produced via anaerobic metabolism. Lactic acid will titrate plasma bicarbonate, resulting in fictitious acidosis. Accumulation of lactate, an unmeasured anion, also will result in an **increased anion gap.**

10. Very mild hypercalcemia. Total calcium is measured by a colorimetric endpoint technique. Therefore, marked lipemia could cause a slight increase in calcium values. In addition, extreme hemolysis also may falsely increase the calcium concentration. Hypercalcemia may accompany renal disease, but is an unusual observation in dogs.

11. Hypophosphatemia. Hypophosphatemia is an unusual laboratory finding. Its presence in this specimen is probably the result of hemolysis. Although erythrocytes contain phosphorus, either hypophosphatemia or hyperphosphatemia may be observed with hemolysis, depending upon the analytical assay used.

12. Increased CK activity. Hemolysis results in increased CK activity in serum. Erythrocytes do not contain CK; however, other substances released from erythrocytes are detected by the coupled analytical reaction used to measure CK activity. The end result is a test value that reflects increased CK activity.

13. Increased amylase activity. Increased amylase activity could be the result of necrotizing pancreatitis and/or decreased renal clearance of plasma enzyme activity.

14. Decreased lipase activity. Hemolysis directly inhibits lipase, resulting in decreased enzymatic activity in the serum specimen.

SUMMARY: The blood and serum specimens for the CBC and biochemical profile are of limited diagnostic use because of hemolysis, lipemia, and delayed shipment to the laboratory. This case demonstrates that the laboratory result is only as useful as the quality of the specimen that is submitted for laboratory analysis. Common causes of artifactual changes in blood and serum laboratory specimens include: (1) failure to collect fasting laboratory specimens (unless hypoglycemia is suspected), (2) *in vitro* hemolysis, (3) failure to separate serum from the clot, (4) improper specimen storage, and (5) delay in conveying the specimen to the laboratory for analysis.

Free ebooks ==> www.Ebook777.com

INDEX

Note: Italicized page locators indicate a figure/photo; tables are noted with a *t*.

A
AALA. *See* American Association for Laboratory Accreditation
AAVLD. *See* American Association of Veterinary Laboratory Diagnosticians
Abrupt water deprivation test, 273–274
Absolute polycythemia, primary and secondary, 41
Absolute reticulocyte count, formula for, 22
Absorption tests, 240
Abyssinian cats, PK deficiency in, 5
Acanthocytes
  description of, 19
  dog, *17*
Accelerated erythrocyte destruction, causes of, intravascular *vs.* extravascular hemolysis, 31*t*
Accreditation, for laboratories, 368
Accuracy, test validity and, 366
Acetylcholine receptor antibodies, myasthenia gravis and, 289
Acid-base balance, exercise and, 155
Acid-base disorders, common, electrolyte patterns in, *164*
Acid-base imbalance
  laboratory differentiation of, 151*t*
  patterns of, 150
Acid-base regulation, 150
Acidosis, feline infectious panleukopenia and, 407
ACT. *See* Activated clotting time
ACTH. *See* Adrenocorticotropic hormone
ACTH concentration, endogenous, measurement of, 322
ACTH test, protocol and interpretation of, 320
Actinomyces sp.
  filamentous beaded bacteria typical of, *335*
Activated clotting time, 132
Activated factor IX, 127
Activated factor X, 127
Activated partial thromboplastin time
  clotting factor activity and, 224
  prolonged, 135
  test, 136
Acute granulocytic leukemia
  blood, cat, *85*
  lymph node, cat, *84*
Acute intravascular hemolytic anemia (case 3), 389–391
  laboratory data, *389–390*
  problems, 390–391
  signalment, history, presenting problems, 389
  summary, 391
Acute lymphoblastic leukemia, blood of horse, *84*
Acute lymphocytic leukemia, 91–92
  definition and occurrence, 91
  differential diagnoses, 92
  laboratory features, 92
Acute myeloblastic leukemia, 96–97
Acute myeloid leukemia, 96–98
  classification, 96
  subgroups, 96–98
Acute necrotizing pancreatitis, secondary hyperlipidemia and, 189
Acute pancreatic necrosis (case 14), 418–420
  laboratory data, *418–419*
  problems, 419–420
  signalment, presenting problems, 418
  summary, 420
Acute phase index, calculation of, 179
Acute phase protein methods, types of, 177
Acute-phase proteins, 178, 179
  assessing herd health status and, 180
  measuring, 179
  saliva as surrogate for blood in assessment of, 179–180
Acute-phase response, 179
Acute renal failure (ethylene glycol toxicosis) (case 18), 430–432
  laboratory data, *430–431*
  problems, 431–432
  signalment, presenting problems, 430
  summary, 432
Acute septic mastitis (case 21), 438–439
  laboratory data, *438*
  problems, summary, 439
  signalment, presenting problems, 438
Acute submassive or massive hepatic necrosis, 225
Acute undifferentiated leukemia, 96
Adenocarcinomas, nasal, 357
Adenosine triphosphate
  creatine kinase and, 283
  Embden-Meyerhof pathway and, 5
Adipokine assays, 187

www.Ebook777.com

Adipokines, obesity and, 190
Adiponectin, obesity and, 190
Adrenal cortex, 317–323
  basic concepts about, 317–318
  diseases of, 318–319
  laboratory evaluation of, 319–323
Adrenal function tests, hypothalamic-pituitary-adrenal axis and, *319*
Adrenal insufficiency with cortical atrophy, laboratory findings in, 323
Adrenocorticotropic hormone, 317, *319*
ADVIA-120, 55
Aelurostrongylus abstrusus larva and eosinophils, *345*
African swine fever virus, thrombocytopenia and, 121
AG. *See* Anion gap
Agglutination
  description of, 18
  tests, 25
Aggregate reticulocytes, in cats, 21–22
AgNOR score, 88
AGP, glycosylation of, degree and nature, 180
AID. *See* Anemia of inflammatory disease
AIHA. *See* Autoimmune hemolytic anemia
Akita
  microcytic erthrocytes in, 14
  microcytosis without anemia in, 40
Alanine aminotransferase
  hepatocellular leakage and, 211
  hepatocellular leakage enzymes and, 213
  muscle and, 284
Alaskan Malamutes, stomatocytosis with macrocytosis in, 15
Albumin
  anion gap and, 153
  concentrations, abnormalities in, 223–224
  measuring, 176
  percentage of, in total serum protein concentration, 173
  serum half-life of, 223
Albumin/globulin (A/G) ratio, calculating, 177
Aldolase, in muscle, 285
Algae, in urine, 271
Alimentary lymphoma, 87, 89
Alkaline phosphatase
  cholestasis and, 212
  induced hepatic enzymes and, 214–215
  urine activity and, 264
Alkaline urine with triple phosphate crystalluria, urea toxicosis and, 461
ALL. *See* Acute lymphocytic leukemia
ALP. *See* Alkaline phosphatase
ALP activity, increased, hyperadrenocorticism and diabetes mellitus, with other complications and, 471
Alpha-globulins, 174, *174*, 175
Alpha granules, 108–109
ALP isoenzymes, types of, 215–216
ALT. *See* Alanine aminotransferase
ALT activity, increased
  artifacts of lipemia, hemolysis, improper sample handling and, 474
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  urea toxicosis and, 460
American Association for Laboratory Accreditation, 368
American Association of Veterinary Laboratory Diagnosticians, 368
American Cocker Spaniels, platelet dense granule defect in, 119
American Eskimo Dog
  methemoglobin reductase deficiency in, 7
  PK deficiency in, 5
AML. *See* Acute myeloid leukemia
Ammonia
  metabolism of, 222
  production of, in gastrointestinal tract, 221
  special handling and measurement of, 221
  in urine, 255
Ammonia tolerance test, 221
Ammonium biurate, in urine sediment, *267*
Ammonium biurate crystalluria, hepatic failure and, 417
Ammonium biurate crystals, in urine sediment, 271
Amoxicillin, renal tubular damage and high dosages of, 260
Ampicillin crystals, in urine, 272
Amylase
  measurement of, 231–232
  serum amylase activity, characteristics of, 232–233
Anal sac, apocrine gland adenocarcinoma of, 300
Analytical method parameters, reference intervals and, 370
Analytical sensitivity, test validity and, 366
Analytical test errors, 366, 367
Anaplasma centrale, 21
Anaplasma marginale, 21
  in cow, *17*
Anaplasma phagocytophilum morulae, in cytoplasm of two neutrophils, *62*
Anaplasma platys, in dog, *17*
Anaplasmataceae spp. bacteria, in leukocytes, 61
Ancylostomiasis, 385
Androgens, Epo release and, 11
Anemia of chronic disorders
  feline infectious peritonitis and, 401
  multicentric lymphoma and, 409
  renal abscess and secondary disseminated intravascular coagulation and, 411
Anemia of chronic renal disease, renal amyloidosis and, 434
Anemia of inflammatory disease, 37–38
Anemia(s)
  from accelerated erythrocyte destruction, 30, 31*t*, 32–35
  from blood loss, 28–30
  in chronic renal disease, 279
  classification, 27–28
    according to bone marrow response, 27–28
    according to major pathophysiologic mechanisms, 28
    according to size and Hb concentration, 27
  clinical signs and physical findings, 26
  defined, 26
  diagnosis, 26–27
  external *vs.* internal hemorrhage, differential features, 29–30
  history, 26
  laboratory findings, 26–27
  microangiopathic, 35
  from reduced or defective erythropoiesis, 35–40
    causes of, 36*t*

Free ebooks ==> www.Ebook777.com

differentiation of, 37–40
general considerations, 36–37
Anestrus, cytologic characteristics of, 360
Anion gap
albumin and, 153
electrolytes and, 151–153
calculations, 152
interpretation of, 152–153
methods of measurement, 151–152
elevated, urea toxicosis and, 460
high with metabolic acidosis, end stage renal disease with uremic pneumonitis and, 464
Anisocytosis
description of, 18
urea toxicosis and, 459
Anorexia
feline infectious peritonitis and, 400
hepatic encephalopathy and, 413
hepatic lipidosis and, 225
Antibiotic-responsive diarrhea, 240
Antibody detection, 24–26
Antibody production, lymphocytes and, 58
Anticoagulation, disorders in, 128–129
Anti-erythrocyte antibodies
detection of, 25–26
antiglobulin (Coombs) test, 25–26, 32
blood cross matching, 25
Antigen detection, 24–26
Antiglobulin (Coombs) test, 25–26, 32
Antiplatelet antibody, 114
Anti-T4 autoantibodies, in dogs, 310
Antithrombin deficiency, 128
Anti-thyroglobulin autoantibodies, 309–310
Aperture closure instruments, 114
Aperture impedance flow automated hematology instruments, 111
API. *See* Acute phase index
Aplastic anemia, causes of, 38
APTT. *See* Activated partial thromboplastin time
Arabian foals, severe combined immunodeficiency disease in, 182
Arterial partial pressure of oxygen, 148
Arterial PCO_2, 168–169
Arterial PO_2, 167–168
Artifacts of lipemia, hemolysis, and improper sample handling (case 35), 472–474
laboratory data, *472–473*
problems, 473–474
signalment, presenting problems, comment, 472
summary, 474
Ascites, in dogs with heart failure, 351
Aspartate aminotransferase
diagnostic significance of, 285, 287
diseases of muscle origin and, 286*t*
hepatocellular leakage and, 211
hepatocellular leakage enzymes and, 214
increased activity of
muscle disease and, 429
urea toxicosis and, 460
muscle and, 284
skeletal muscle disease in birds and, 287
Aspergillus sp., nasal infections and, 357
AST. *See* Aspartate aminotransferase
Asynchronous nuclear maturation, 60
AT activity, 137
Ataxia, circling, hepatic encephalopathy and, 413
Atoxoplasma sp., in cytoplasm of avian lymphocytes, *63*
ATP. *See* Adenosine triphosphate
A-type natriuretic peptides, 288
AUL. *See* Acute undifferentiated leukemia
Australian Shepherds, Pelger-Huet anomaly in, 60
Autoagglutination, immune-mediated anemia, in dog, *17*
Autoimmune hemolytic anemia, 32
Autoimmune hemolytic anemia and thrombocytopenia (case 2), 386–388
laboratory data, *386–387*
problems, 387–388
signalment, medical history, presenting problems, 386
summary, 388
Automated cell counters, 58
Automated differential leukocyte counts, 55
Automated platelet counts, 111
Avian coagulation and fibrinolysis, 132
Avian erythrocytes, morphology, 18
Avian heterophil, monocyte, thrombocyte, *46*
Avian heterophils, 45
morphology of, 49
normal and toxic, *47*
toxic changes in, 60
Avian lymphoma, anatomic, functional, and laboratory features, 90
Avian species, erythropoiesis in, 9
Avian thrombocytes, morphology, 109
Avian urine collection, 254
Azotemia
acute intravascular hemolytic anemia and, 390
acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
acute salmonellosis and, 398
artifacts of lipemia, hemolysis, and improper sample handling and, 473
defined, 273
end stage renal disease with uremic pneumonitis and, 464
hyperadrenocorticism and diabetes mellitus, with other complications and, 470
muscle disease, myoglobinuric nephrosis and, 429
nephrosis and perirenal hemorrhage and, 441
postrenal, 276
feline lower urinary tract disease and, 437
prerenal, 275–276
acute pancreatic necrosis and, 419
pyometra and, 403–404
renal, 276
renal amyloidosis and, 434
renal failure and, 423
Azurophilic granules, 45

B
Babesia bigemina, 21
Babesia bovis, 21
Babesia caballi, 21
Babesia canis, *17*, 21
Babesia cati, 21

www.Ebook777.com

Babesia equi, 21
Babesia felis, 21
Babesia gibsoni, 21
Babesia motasi, 21
Babesia ovis, 21
Bacilli, 270
Bacteria
  in urine sediment, *266*, 270–271
    identifying features, 270–271
    significance of bacteriuria, 271
BALT. *See* Bronchial-associated lymphoid tissue
Band, granulopoiesis, 49, *49*
Barbiturates, hyporesponsive platelets and, 120
Base excess, 149
Basenjis
  basal serum T4 and fT4 concentrations in, 309
  glucosuria in, 260
  PK deficiency in, 5
Basopenia, 77
Basophil colony-forming units, *8*
Basophilia
  causes of, 76*t*
  description and mechanisms of, 76–77
Basophilic leukemia, blood of dog, *86*
Basophilic stippling
  description of, 20
  lead toxicosis, dog, *17*
  regenerative anemia, cow, *17*
Basophils, 45, 54–55
  canine, *47*
  degranulated, 61
  equine, *47*
  feline, *47*, *48*
  function, 55
  mammalian, 55
  morphology, 54–55
  production and kinetics, 55
Basset Hound thrombopathia, 118
BE. *See* Base excess
Beagle, PK deficiency in, 5
Belgian Tervuren dogs, inherited familial neutropenia in, 73
Bence Jones proteinuria, 94, 258
Benzene, thrombocytopenia and, 121
Beta-globulins, 174, *174*, 175
Beta-glucuronidase, urine activity and, 264
Beta-lactam antibiotics, hyporesponsive platelets and, 120
BFU-E. *See* Erythroid burst-forming units
BFU-MK. *See* Burst-forming unit megakaryocyte
Bicarbonate, fractional clearance of, 279
Bicarbonate ion, 148
Bile acids
  increased/decreased concentration of, 221
  metabolism of, *220*
  testing, 219, 221
  uptake and excretion, hepatic function evaluation and, 212
Bile peritonitis
  effusions and, 351
  intracellular and free bile pigment in, *344*
Bile salts, 219
Biliary obstruction, biochemical profile, 228*t*
Biliprotein, 218
Bilirubin
  conjugated, 217, 218, 219, 261
  with hyaline cast, spermatozoa, and leukocytes, in urine sediment, *267*
  interpretation of positive test result, 261
  measurement of, 218, 261
  metabolism of, 217, *217*
  physiology of, 261
  types of, 217–218
  unconjugated, 217, 219, 261
  uptake and excretion, hepatic function evaluation and, 212
Bilirubin concentration, urea toxicosis and, 461
Bilirubin crystals, in urine sediment, 271
Bilirubinuria, 218, 261
  acute pancreatic necrosis and, 419
  hepatic failure and, 417
Biliverdin, 219
  erythrocyte destruction and, 11, 12
  heme catabolism in birds and, 261
Biliverdinemia, 219
Biopsies, bone marrow, 24
Birds
  age-related changes in plasma proteins in, 173
  biliverdin in, 219
  biuret method for total protein measurement in, 176
  blood glucose values in, *vs.* in mammals, 191
  BUN interpretation in, 276–277
  corticosteroid-induced heterophilia in, 68
  filamentous organisms typical of megabacteria of, *335*
  GGT and biliary disease in, 216
  glucose metabolism in, 193
  glucosuria in, 260
  heteropenia in, 71–73, 72*t*
  heterophilia in, 64–71
  hyperuricemia and renal disease in, 278
  lymphoid neoplasia in, 83
  lymphoma in, 90
  megabacteria of, 338
  neutrophilia and heterophilia of inflammation/infection in, 71
  reticulocytes in, 21
  thyroid function testing in, 312
  urinalysis, not useful in, 253
  urine osmolality measurement in, 257
  urine transparency in, 255
  xanthomas in, 338
Birman cat neutrophil abnormality, 64
Biuret method, for total protein measurement, 175, 176
Blastomyces dermatitidis yeasts, *334*, 338
Bleeding time, 115
Blister beetle toxicosis, in horses, hypocalcemia and, 302
Blood, neutrophil kinetics in, 51
Blood cross matching, detection of anti-erythrocyte antibodies and, 25
Blood gases
  analysis of
    arterial partial pressure of oxygen, 148
    base excess, 149
    bicarbonate ion, 148–149

Free ebooks ==> www.Ebook777.com

hydrogen ion concentration, 148
instrumentation, 147
partial pressure of carbon dioxide, 148
sample management, 147–148
determination of, 147–149
acid-base regulation, 150
blood gas analysis, 147–149
pulse oximetry, 148, 149–150
total carbon dioxide content, 149
Blood glucose concentration
maintenance of, 193
measuring, 195
Blood glucose meters, 194
Blood loss, 28–29
acute, characteristics of, 28–29
causes of, 28*t*
chronic, characteristics of, 29
Blood pH, variations of, 154–155
Blood smears
evaluation, leukocytes, 59–61, 64
peripheral, staining and examination of, 15–16
platelet evaluation/determination from, 112–113
Blood urea nitrogen, 274–277
in birds, interpretation of, 276–277
increased concentration
hyperthyroidism and, 454
interpretation of, 275–276
urea toxicosis and, 460
measurement methods, 275
metabolism concepts, 274–275
urea excretion, 275
"Blue eye," 188
BLV. *See* Bovine leukemia virus
B lymphocytes, 57
BMBT. *See* Buccal mucosal bleeding time
Body cavity effusions, 347–352
classification of, 349–350
fluid analysis, 348–349
color, 348
cytology, 348–349
nucleated cell counts, 348
protein determination, 348
general considerations, 347–348
mesothelial cells, *343*
specific conditions, 350–352
Bone ALP isoenzyme, 216
Bone marrow
hematopoietic and leukemic cells in, *10*
neutrophil release from, 51
Bone marrow examination, 23–24
indications for, 23
stained bone marrow smear, 24
technique, 23–24
Bone marrow failure, primary and secondary, anemia from reduced or defective erythropoiesis and, 37
Bone marrow hyperplasia, chronic hemorrhagic anemia and, 396
Bone marrow neutrophils
maturation and storage compartment, 50
proliferation or mitotic compartment, 50
Bone marrow panhypoplasia, 121
Borzoi, methemoglobin reductase deficiency in, 7
Botrocetin assays, 116
Bovine erythrocytes
morphology of, 16
platelets and, *17*
Bovine leukemia virus, 91
Bovine lymphoma
anatomic, functional, and laboratory features, 91
role of bovine leukemia virus in, 91
Bovine renal failure, 280
Bovine thrombopathia, 118
Bovine viral diarrhea, thrombocytopenia and, 121
Bowel obstruction, serum lipase activity and, 233
Briard dogs, primary hypercholesterolemia of, 188
Bromosulphalein, 223
Bronchial-associated lymphoid tissue, 57, 58
Brown urine, acute intravascular hemolytic anemia and, 391
BSP. *See* Bromosulphalein
BT-PABA absorption test, exocrine pancreatic insufficiency and, 239
B-type natriuretic peptides, 288
Buccal mucosal bleeding time, 115
prolonged, disseminated intravascular coagulation and, 411
von Willebrand's disease and, 457
Budgerigars, xanthomas in, 338
BUN. *See* Blood urea nitrogen
Burmese cats, lipid aqueous in, 188
Burst-forming unit megakaryocyte, 109

C
Cairn Terrier, PK deficiency in, 5
Calcitonin, production of, 297
Calcium, 297–299
laboratory abnormalities in, 299–304
major disorders involving, *296*
measurement of, 298–299
colorimetric methods, 298
ion-selective electrodes, 298
urinary calcium excretion, 298–299
serum calcium concentration and dietary intake of, 298
total serum calcium, forms of, 297
Calcium carbonate, with hyaline cast, spermatozoa, and leukocytes, in urine sediment, *267*
Calcium channel blockers, hyporesponsive platelets and, 120
Calcium oxalate dihydrate, in urine sediment, *268*
Calcium oxalate dihydrate crystals, in urine, 272
Calcium oxalate monohydate, in urine sediment, *267*
Calcium oxalate monohydrate crystalluria, acute renal failure (ethylene glycol toxicosis) and, 431
Calcium oxalates, in urine sediment, 271–272
Calcium-sensing receptor, 295
CalDAG-GEFI platelet disorders, 118
CALP. *See* Corticosteroid ALP isoenzyme
CALT. *See* Conjunctival-associated lymphoid tissue
Calves, hypomagnesemia in, 304
Camelidae erythrocytes, morphology of, 18
Camelidae family, elliptical erythrocytes in members of, 40
cAMP. *See* Cyclic adenosine monophosphate

www.Ebook777.com

Campylobacter spp., 246
Cancer, neoplasms arising from hematopoietic cells and, 83
Candida spp.
in urinary sediment, *265*
yeasts, 338, *346*
Canine acute lymphocytic leukemia, lymphoblasts in, *10*
Canine bladder tumor antigen test, 264
Canine distemper inclusions, in neutrophil and erythrocytes, *62*
Canine distemper virus, in leukocytes, 61
Canine eosinophil and basophil, *47*
Canine erythrocytes
morphology of, 16
platelets and, *17*
Canine Franconi-like syndrome, glucosuria and, 260
Canine granulocytic ehrlichiosis, 61
Canine lymphoma, 88–89
anatomic and functional features, 88–89
laboratory features, 89
Canine mature small lymphocyte, *48*
Canine monocytic ehrlichiosis, 61
Canine myeloblastic leukemia, promyelocytes in, *10*
Canine neutrophils, *46*
Histoplasma capsulatum yeasts in cytoplasm of, *62*
Canine parvoviral enteritis, neutropenia and, 72
Canine plasma cell myeloma, plasma cells in, *10*
Canine reactive lymphocyte or immunocyte, *48*
Canine reticulocytes, *17*
Canine transmissible venereal tumor, *337*
Capillaria plica, in urine specimens, 271
Capillaria plica ovum, in urinary sediment, *265*
Capillary electrophoresis, 177
Caprine erythrocytes, morphology of, 18
Carboxylated factors (II, VII, IX, and X), 125
Carcinomas, nasal, 357
Cardiac disease
hepatic disorders and, biochemical profile, 228*t*
troponins and, 183
Cardiac troponins, muscle disease and, 287–288
Case studies, 383–474
acute intravascular hemolytic anemia (red maple toxicosis), 389–391
acute pancreatic necrosis, 418–420
acute pulmonary hemorrhage and edema, 445–446
acute renal failure (ethylene glycol toxicosis), 430–432
acute salmonellosis, 397–399
acute septic mastitis, 438–439
artifacts of lipemia, hemolysis, and improper sample handling, 472–474
autoimmune hemolytic anemia and thrombocytopenia, 386–388
chronic hemorrhagic anemia (iron-lack anemia), 394–396
early primary hypothyroidism, 465
end stage renal disease with uremic pneumonitis, 462–464
estrogen-induced pancytopenia (Sertoli cell tumor), 392–393
exocrine pancreatic insufficiency, diabetes mellitus, and chronic renal failure, 421–423
feline infectious panleukopenia (parvovirus infection), 405–407
feline infectious peritonitis, 400–401
feline lower urinary tract disease, 436–437
hemorrhagic anemia (ancylostomiasis), 385
hepatic encephalopathy (hepatic fibrosis), 413–414
hyperadrenocorticism, 450–452
hyperadrenocorticism and diabetes mellitus with ketoacidosis, hyperosmolality, necrotizing pancreatitis, and UTI, 468–471
hyperthyroidism, 453–455
inflammatory bowel disease, 424–426
multicentric lymphoma, 408–409
muscle disease, myoglobinuric nephrosis, 427–429
nephrosis and perirenal hemorrhage, 440–442
portosystemic venous shunt with hepatic atrophy, 415–417
possible glucocorticoid suppression of thyroid function, 466
primary hypothyroidism (and phenobarbital administration), 467
pyometra, 402–404
renal abscess and secondary disseminated intravascular coagulation, 410–412
renal amyloidosis, 433–435
right abomasal displacement, 443–444
rodenticide toxicosis, 447–449
urea toxicosis, 458–461
von Willebrand's disease, 456–457
CaSR. *See* Calcium-sensing receptor
Casts
types of, 270
in urine sediment examination, 270
Catecholamines
eosinopenia and, 75
glycogenolysis and, 191
Catheterized urine specimens, 253–254
Cats
bilirubinuria in, 261
chronic renal disease and hypocalcemia in, 302
continuous glucose monitoring devices used for, 194
corticosteroid-induced neutrophilia in mammals/heterophilia in birds and, 67–68
diagnosis of exocrine pancreatic insufficiency in, 238–239
erythroleukemia in, 98
estimates of regeneration for punctate reticulocytes in, 23
glucosuria in, 260
hemophilia B in, 127
hypercalcemia in, 299
hypereosinophilic syndrome in, 74
hyperthyroidism in, 453–455
intestinal isoenzyme of ALP in, 216
lymphoma in, 89–90
metabolic acidosis and renal failure in, 280
neutrophilia and heterophilia of inflammation/infection in, 70
obesity in, 190
pancreatitis in, 234, 237
diagnostic steps for, 237–238

Free ebooks ==> www.Ebook777.com

parvovirus infection in, 405–407
performing TRH stimulation test in, 315
platelet counts for, 111
polycythemia vera in, 100
reticulocytes in, 21–22
Cattle
cold hemoglobinuria of, 35
corticosteroid-induced neutrophilia in mammals/heterophilia in birds and, 68
high serum LDH activity and lymphoma in, 287
hypomagnesemia in, 304
ketonuria and ketosis of, 261
lymphoma in, 91
neutrophilia and heterophilia of inflammation/infection in, 70
parturient paresis and hypocalcemia in, 302
polycythemia vera in, 100
renal disease and hypocalcemia in, 280
variability of erythrocyte antigens in, 25
Cavalier King Charles Spaniels
macrothrombocytopenia in, 119
thrombocytopenia in, 112
CBA. *See* Collagen-binding activity assay
CD34+ cells, 7
Cell counters, automated, 13, 14, 58
Cellular casts, 270
Centrilobular hepatic lesions caused by hypoxia, 224–225
clinical features and characteristics of, 224
laboratory findings on, 224–225
Cerebrospinal fluid, 354–356
cytologic examination, 355–356
general considerations, 354
gross appearance, 354
with pleocytosis and *Cryptococcus neoformans* yeasts, *344*
protein, 355
special tests, 356
total nucleated cell count, 355
Cerebrospinal fluid CK activity, central nervous system disease and, 287
Ceruloplasmin, 180
iron transport and, 5
CFU-Bas. *See* Basophil colony-forming units
CFU-E. *See* Erythroid colony-forming units
CFU-G. *See* Granulocyte colony-forming units
CFU-GEMM. *See* Myeloid stem cell
CFU-GM. *See* Granulocyte/monocyte colony-forming units
CFU-M. *See* Monocyte colony-forming units
CFU-Meg. *See* Megakaryocytic colony-forming units
CFU-MK. *See* Colony-forming-unit-megakaryocyte
CGL. *See* Chronic granulocytic leukemia
Chédiak-Higashi syndrome, 64, 118
Chelated or complex calcium, 297
Chemistry reference intervals, 374–375*t*
Chemokine receptor 4, 110
Chemotaxis, neutrophils and, 51
Chemotherapeutic agents, thrombocytopenia and, 121
Chesapeake Bay Retrievers, Type 3 VWD in, 118
Chihuahua
methemoglobin reductase deficiency in, 7
PK deficiency in, 5
Chlamydophila psittaci, in cytoplasm of avian monocyte, *63*
Chloramphenicol, inhibition of heme synthesis and, 3
Cholangiohepatitis, clinical features, characteristics, and laboratory findings on, 226
Cholangitis
biochemical profile, 228*t*
clinical features, characteristics, and laboratory findings on, 226
Cholestasis, 218
acute pancreatic necrosis and, 419
defined, 212
Cholesterol, 183
lipoprotein profiles and, 185
Cholesterol crystals, in urine, 272
Cholesterol esters, 183
Cholesterol ester-transfer protein, lack of, 185
Chondrosarcomas, nasal, 357
Chow Chow
microcytic erthrocytes in, 14
microcytosis without anemia in, 40
Christmas factor, 124
Chromogenic technique, Protein C and, 137
Chronic basophilic leukemia, 99
Chronic eosinophilic leukemia, 99
Chronic granulocytic leukemia, characteristics of, 99
Chronic hemorrhagic anemia (case 5), 394–396
laboratory data, *394–395*
problems, 395–396
signalment, presenting problems, 394
summary, 396
Chronic lymphocytic leukemia, 92–93
blood, horse, *84*
definition and occurrence, 92
differential diagnoses, 93
laboratory features, 92–93
Chronic monocytic leukemia, 100
Chronic myeloid leukemia, 99–100
definition, 99
subgroups, 99–100
Chronic myelomonocytic leukemia, 100
Chronic renal disease
hyperthyroidism and, 313
hypocalcemia and, 302
Chylomicra, 185
Chylous effusions, 350
Ciliated epithelial cells and alveolar macrophages, *345*
Circovirus, heteropenia in birds and, 72
Citrated plasma clotting tests, 133–136
activated partial thromboplastin time, 135
methods, 134–135
prothrombin time, 135–136
Russell viper venom test, 136
sample management, 133–134
specific factor analysis, 136
thrombin time, 136
CK. *See* Creatine kinase
CK-BB, 283
CK-MB, 283
CK-MM, 283
Classification of Tumours of Haematopoietic and Lymphoid Tissues (WHO), 96
"Clean catch" urine collection, 253

www.Ebook777.com

CLL. *See* Chronic lymphocytic leukemia
Clostridium sordelli, equine hyperammonemia and, 245
Clostridium spp.
  in inflammatory lesions, 338
  testing for, malabsorption and, 240
Clot retraction evaluation scale, *116*
Clot retraction test, 115–116
Clotting factor activity, liver and, 224
Cluster of differentiation (CD) markers, 83
CML. *See* Chronic myeloid leukemia
Coagulation
  avian, 132
  defined, 107
  laboratory evaluation of, 132–138
  mechanisms and disorder of extrinsic pathway of, 126
  mechanisms and disorders of common pathway of, 128–129
  mechanisms of, 123–126
Coagulation factor deficiency, rodenticide toxicosis and, 449
Cobalamin, small intestinal bacterial overgrowth and, 240–241
Cobalamin concentration, malassimilation diseases and typical laboratory changes in, 241*t*
Cobalamin measurements, exocrine pancreatic insufficiency and, 239
Cocci, 270
Coccidia, 339
Coccidioides immitis yeasts, 338
  surrounded by inflammatory cells, *335*
Cockatiels, xanthomas in, 338
Cocker Spaniel-Poodle cross, methemoglobin reductase deficiency in, 7
Cocker Spaniels, PFK deficiency in, 7
Codocytes, description of, 19–20
COLA, 368
Collagen-binding activity assay, 116
Colloid osmometry, 182–183
Colloid osmotic pressure
  serum or plasma
    determination of, 182–183
    estimation of, 183
    physiologic considerations, 182
Colony-forming-unit-megakaryocyte, 109
Colony-stimulating factors
  monocytes as major source of, 53
Colony-stimulating factors, granulopoiesis and, 49
Colorimetric methods
  for calcium measurement, 298
  for phosphorus measurement, 299
Colorimetric reagent strips, reading, 194
Colostral antibody, detecting failure of passive transfer of, in foals and calves, 176–177
Colostrum, mare, antibodies in, 25
Commercial laboratories, quality control in, 367–368
Complete blood count
  megaesophagus and, 244
  pancreatic inflammation and necrosis and, 235
  testing for, malabsorption and, 240
Confirmatory tests, 378
Congenital portosystemic shunts
  chronic, progressive, 228*t*
  clinical features, characteristics, and laboratory findings on, 227
Conjugated bilirubin, 217, 218, 219, 261
Conjunctival-associated lymphoid tissue, 57
Coomassie Brilliant Blue RG 230, dye-binding method for total protein measurement, 175
Coombs tests. *See* Antiglobulin (Coombs) tests
Coonhounds, Pelger-Huet anomaly in, 60
COP. *See* Colloid osmotic pressure
Copper deficiency, iron deficiency anemia and, 40
Copper reduction tablet method, glucosuria and, 260
Corrected reticulocyte percentage, formula for, 22
Corticosteroid administration, serum lipase activity and, 234
Corticosteroid ALP isoenzyme, 215–216
Corticosteroid hepatopathy
  biochemical profile, 228*t*
  clinical features, characteristics, and laboratory findings on, 226
Corticosteroid-induced neutrophilia in mammals/heterophilia in birds, 66–68
  eosinopenia, causes, 67
  leukogram findings, 66–67
  lymphopenia, causes, 67
  mechanisms, 67
  possible clinical findings, 67
  species characteristics
    birds, 68
    cats, 67–68
    cattle, 68
    dogs, 67
    horses, 68
Corticosteroids, gluconeogenesis and, 192
Cortisol concentration, high baseline, hyperadrenocorticism and diabetes mellitus, with other complications and, 471
Coumarin toxicosis, 447–449
Cows
  acid-base status of, 280
  acute septic mastitis in, 438–439
  glucosuria in, 260
  nephrosis and perirenal hemorrhage in, 440–442
  right abomasal displacement in, 443–444
  urea toxicosis in, 458–461
CRD. *See* Chronic renal disease
C-reactive protein, 179
  pancreatic inflammation and necrosis and, 236
Creatine kinase
  diagnostic significance of, 285, 287
  diseases of muscle origin and, 286*t*
  increased activity, urea toxicosis and, 460
  muscle and principal isoenzymes of, 283–284
  skeletal muscle disease in birds and, 287
Creatinine, metabolism of, 277
CRH, *319*
Cross match tests, detection of anti-erythrocyte antibodies and, 25
Cryoglobulinemia, 94
Cryptococcus neoformans yeast, *334*, 338
Cryptosporidiosis, testing for, malabsorption and, 240

Free ebooks ==> www.Ebook777.com

Crystalluria, 271–272
CSFs. *See* Colony-stimulating factors
cTn1, 183
cTnT, 183
Curschmann's spirals, *345*
Cushing's syndrome, 317
Cutaneous lymphoma, 87
Cut-offs
  choosing, 376
  decreased value and, *378*
  defined, 373
  increased value and, *379*
  receiver operator characteristic (ROC) curve, *379*
Cyanide-free hemoglobinhydroxylamine complex method, Hb concentration and, 13
Cyanmethemoglobin technique, Hb concentration and, 13
Cyclic adenosine monophosphate, production of, 107
Cyclic hematopoiesis, 73, 119
Cyclooxygenase inhibitors, hyporesponsive platelets and, 120
Cystine crystals, in urine, *268*, 272
Cystocentesis specimens, 254
Cytauxzoon felis, 21
Cytokines, 49
Cytologic examination, for synovial fluid analysis, 353
Cytology, 331–361
  body cavity effusions, 347–352
    bile peritonitis, 351
    chylous effusions, 350
    classification of, 349–350
    egg yolk peritonitis, 351–352
    exudates, 350
    feline infectious peritonitis, 350
    fluid analysis, 348–349
    general considerations, 347–348
    heart failure, 350–351
    hemorrhagic, 351
    neoplasms, 351
    specific conditions, 350–352
    uroperitoneum, 351
  cerebrospinal fluid, 354–356
    cytologic examination, 355–356
    general considerations, 354
    gross appearance, 354
    protein, 355
    special tests, 356
    total nucleated cell count, 355
  diagnosis of digestive disorders and, 246
  equine endometrial, method of collection and cytologic examination, 361
  inflammatory lesions, 333, 338–339
  lymph nodes, 342, 347
    hyperplastic/reactive, 347
    lymphadenitis, 347
    lymphoma, 347
    metastatic neoplasia, 347
    normal, 342, 347
  neoplasms, 340–342
  noninflammatory, nonneoplastic lesions, 339–340
  respiratory system, 356–359
    nasal exudates and masses, 356–358
    tracheo-bronchoalveolar cytology, 358–359
  specimen collection and handling, 331–333
    fine-needle aspiration, 331–332
    fluids, 332
    smear preparation, 332–333
    stab method/nonaspiration fine-needle biopsy, 332
    swabs, 332
    tissue imprints, 331
    tissue scrapings, 331
  synovial fluid, 352–354
    appearance, 352
    cytologic examination, 353–354
    mucin clot test, 352–353
    nucleated cell count, 353
    protein, 353
    viscosity, 352
  vaginal, 360–361
    cell collection, 360
    cytologic characteristics of estrus cycle, 360
    normal cells, 360
    reproductive diseases, 360–361
Cytoplasmic basophilia, 60
Cytoplasmic enzymes, evaluation of, 213–214
Cytoplasmic vacuolation, 60
Cytotoxicity, 58

D
Dacryocytes, description of, 19
Dalmations, uric acid crystals in, 272
D-dimer, 137
DEA. *See* Dog erythrocyte antigen
Defective erythropoiesis
  anemia from
    causes of, 36*t*
    differentiation of, 37–40
    general considerations, 36–37
Degranulated eosinophils, 61
  canine, *47*
Dehydration
  evaluation of, 145
  relative polycythemia and, 41
DELAT test, 26, 32
Dendritic cell neoplasms, 100–101
  definition, 100–101
  subgroups, 101
Dense granules, 109
Devon Rex cats, inherited vitamin-K-dependent multifactor coagulopathies in, 129
Dexamethasone suppression test
  high-dose, 321
  low-dose, 320–321
Dextrose-containng parenteral fluids, hyperglycemia and, 192
Diabetes control devices, 194
Diabetes mellitus, 193, 315
  hepatic lipidosis and, 225
  hyperadrenocorticism and, 468–471
  hypocalcemia and, 301
  ketonuria and, 261
  laboratory assessment of endocrine pancreas and, 316–317
  secondary hyperlipidemia and, 189

www.Ebook777.com

Diarrhea, clinical investigation of, 240
Diazotization method, for bilirubin measurement, 261
DIC. *See* Disseminated intravascular coagulation
Diestrus, cytologic characteristics of, 360
Diff-Quick, 16
Diffuse pattern of lymphoma, 87
Digestive system, 231–246
  cytology, 246
  equine hyperammonemia, laboratory detection of, 245–246
  exocrine pancreas, 231
  malassimilation attributable to malabsorption, laboratory detection of, 239–243
  malassimilation attributable to maldigestion, laboratory detection of, 238–239
  megaesophagus, laboratory detection of, 243–244
  pancreatitis, laboratory detection of, 231–238
  protein-losing enteropathy, laboratory detection of, 244–245
Diltiazem, hyporesponsive platelets and, 120
Diluted samples technique, electrolytes and anion gap and, 152
Dioctophyma renale, in urine specimens, 271
Dioctophyma renale ova, in urinary sediment, *266*
Dipetalonema reconditum, 21
Dipstick ELISA test, for detecting IgG, 177
Dipstick tests, urine protein quantitation and, 257
Direct-reading bilirubin, 218
Dirofilaria immitis, 21
  in urine specimens, 271
Disseminated intravascular coagulation
  hyporesponsive platelets and, 120
  schistocytes and, 19
  secondary, renal abscess and, 410–412
Disseminated intravascular coagulation syndrome, acquired deficiency of nonenzymatic factors in, 128
Distemper inclusions, in canine erythrocytes, description of, 21
Divalent metal transporter 1, 5
D-lactate, 200
DMT1. *See* Divalent metal transporter 1
Dogs
  absolute reticulocyte count and estimated degrees of regeneration for, 22
  acquired von Willebrand disease in, 118
  ACTH test and, 320
  acute pancreatic necrosis in, 418–420
  acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) in, 445–446
  acute renal failure (ethylene glycol toxicosis) in, 430–432
  anti-thyroglobulin autoantibodies in, 309
  artifacts of lipemia, hemolysis, and improper sample handling in, 472–474
  autoimmune hemolytic anemia and thrombocytopenia in, 386–388
  chronic hemorrhagic anemia in, 394–396
  chronic renal disease and hypocalcemia in, 302
  continuous glucose monitoring devices used for, 194
  corticosteroid-induced neutrophilia in mammals/heterophilia in birds and, 67
  detecting transitional cell carcinoma antigens in urine of, 264
  diagnosis of exocrine pancreatic insufficiency in, 238–239
  early primary hypothyroidism in, 465
  end stage renal disease with uremic pneumonitis in, 462–464
  erythrocyte antigens and, 25
  erythropoietin administration in, 121
  estrogen-induced pancytopenia in, 392–393
  exocrine pancreatic insufficiency, diabetes mellitus and chronic renal failure in, 421–423
  exocrine pancreatic insufficiency in, 238
  glucosuria in, 260
  granulomatous disease and hypercalcemia in, 301
  hemorrhagic anemia in, 385
  hyperadrenocorticism and diabetes mellitus in, with ketoacidosis, hyperosmolality, necrotizing pancreatitis, and UTI, 468–471
  hyperadrenocorticism in, 450–452
  hyperadrenocorticism in, serum alkaline phosphatase activity and, 322
  hypercalcemia and renal disease in, 300
  hypercalcemia in, 299
  hypoalbuminemic, 298
  hypothyroid, hypercholesterolemia in, 313
  hypothyroid, serum TSH concentrations in, 309
  inflammatory bowel disease in, 424–426
  influence of breed on T4 and fT4 concentrations in, 308–309
  intestinal isoenzyme of ALP in, 216
  lymphoma in, 88–89
  megaesophagus in, 243
  metabolic acidosis and renal failure in, 280
  multicentric lymphoma in, 408–409
  neutrophilia and heterophilia of inflammation/infection in, 70
  obesity in, 190
  pancreatitis in, diagnostic steps for, 237
  pericardial effusions in, 351
  polycythemia vera in, 100
  primary hypothyroidism (and phenobarbital administration) in, 467
  primary hypothyroidism in, comparing single/multiple analytes for sensitivity, specificity, and accuracy in, 311*t*
  pure megakaryocytic hypoplasia in, 121
  pyometra in, 402–404
  renal abscess and secondary disseminated intravascular coagulation in, 410–412
  renal amyloidosis (nephrotic syndrome) in, 433–435
  reticulocytes in, 21
  rodenticide (coumarin) toxicosis in, 447–449
  serum CK activity in, 284
  serum lipase activity in, 233
  small intestinal malabsorptive disease in, 240
  von Willebrand's disease in, 456–457
Döhle bodies, 60
Drentse-Patrijshond, stomatocytosis with macrocytosis in, 15
Drug-induced thrombocytopenia, 122

Free ebooks ==> www.Ebook777.com

Ducks, urine transparency in, 255
Dutch Kooikers, Type 3 VWD in, 118
Dye-binding method, for total protein measurement, 175
Dye clearance tests, 223
Dyserythropoiesis
  anemia, maturation abnormalities and, 37
  in English Springer Spaniels, 40
Dysproteinemias. *See* Protein abnormalities (dysproteinemias)
Dysthrombocytopoiesis, 123
Dystrophin, muscular dystrophy and, 289

E
Eccentrocytes
  description of, 20
  onion toxicosis, dog, *17*
Echinocytes
  description of, 19
  dog, *17*
EctoADPase release, 108
EDTA
  automated platelet counts and, 111
  fluid collection and, 332
  monocytes and, 52
EDTA-induced platelet clumping, 112
Effusions
  bile peritonitis and, 351
  chylous, 350
  classification of, 347, 349–350
  feline infectious peritonitis as cause of, 350
  hemorrhagic, 351
  pericardial, 351
  uroperitoneum and, 351
EGF. *See* Epidermal growth factor
Egg yolk peritonitis, effusions and, 351–352
Ehrlichia canis, 61
  hyporesponsive platelets and, 120
Ehrlichia weingii, 61
Ehrlichia weingii morulae, in cytoplasm of canine neutrophil, *62*
Ehrlichiosis, thrombocytopenia and, 121
Ehrlich's diazo reaction, 262
Electrolyte clearance, quantifying, 279
Electrolyte concentrations, variations of, 154–155
Electrolytes
  anion gap and, 151–153
    calculations, 152
    interpretation of, 152–153
    methods of measurement, 151–152
  disorders of, 156
  exercise and, 155
  osmolality and, 146
ELISA, 177
  anti-thyroglobulin autoantibodies and, 309
Elliptocytes, description of, 19
Embden-Meyerhof pathway, erythrocyte metabolism and, 5
Endocrine pancreas, 315–317
  laboratory assessment of, 316–317
Endocrine pancreatic hormones, basic concepts of, 315–316
Endocrine system, 295–323
  adrenal cortex, 317–323
  endocrine pancreas, 315–317
  laboratory abnormalities in calcium, phosphorus, and magnesium imbalance, 299–304
  overview of, 295, 297–299
  parathyroid gland, calcium, phosphorus, magnesium and, 295
  thyroid function, 304–315
Endogenous creatinine clearance, calculating, 278
Endogenous lipids, 184
Endometrial cytology, *346*
Endometrial epithelial cells and free cilia, *346*
Endothelial cells, 107
  antithrombotic properties of, 107–108
  procoagulant properties that follow stimulation of, 108
Endothelium, 107
  damage to, 108
End stage renal disease with uremic pneumonitis (case 30), 462–464
  laboratory data, *462–463*
  problems, 463–464
  signalment, medical history, presenting problems, radiographic findings, 462
  summary, 464
Endurance exercise, acid-base balance, electrolytes and, 155
English Springer Spaniels
  dyserythropoiesis in, 40
  PFK deficiency in, 7
Enhanced platelet function, 120–121
Enteritis, serum lipase activity and, 233
Enterocolitis, hyperlipidemia secondary to, 189
Enzymatic factors, 124–125
Enzyme activity, in urine, 264
Eosinopenia
  corticosteroid or "stress" leukogram and, 236
  description and mechanisms of, 75–76
  hyperadrenocorticism and, 451
  neutrophilia and heterophilia of inflammation and, 68
Eosinophilia
  causes of, 75*t*
  description and mechanisms of, 74
  hyperthyroidism and, 454
Eosinophilic inflammation, 359
Eosinophilic leukemia, blood of horse, *86*
Eosinophilic myeloproliferative disease, bone marrow of horse, *86*
Eosinophils, 45, 54, *346*
  canine, *47*
  cytology, body cavity effusions, 349
  degranulated, 61
  equine, *47*
  function, 54
  lymphadenitis and, 347
  morphology, 54
  porcine, *47*
  production and kinetics, 54
Eperythrozoon spp., 21
Eperythrozoon wenyoni, 21
EPI, serum folate concentrations and, 242

www.Ebook777.com

Epicellular parasites, 21
Epidemiology, basic, 373, 376–381
Epidemiology 2 × 2 contingency table, 377*t*
Epidermal growth factor, 111
Epinephrine, glycogenolysis and, 191
Epithelial cells
  ciliated, *345*
  endometrial, *346*
  noncornified, *346*
  in urine sediment, 264, *265*, 269
Epithelial tumors, 340–341
Equine endometrial cytology, method of collection and cytologic examination, 361
Equine eosinophil and basophil, *47*
Equine erythrocytes
  morphology of, 16
  in rouleaux and platelets, *17*
Equine hepatic lipidosis syndrome, of pony mares and horses, 189
Equine hyperammonemia, 245–246
Equine infectious anemia, thrombocytopenia and, 121
Equine lymphoma, 90–91
  anatomic and functional features, 90
  laboratory features, 91
Equine neutrophils, *46*
Erythremic myelosis, blood of cat, *85*
Erythrocyte deformability, decreased, examples of, 32
Erythrocyte destruction, accelerated, causes of, 31*t*
Erythrocyte glutathione peroxidase activity, selenium deficiency and, 289
Erythrocyte morphology, 16–21, *17*
  agglutination, 18
  anisocytosis, 18
  basophilic stippling, 20
  distemper inclusions in canine erythrocytes, 21
  eccentrocytes, 20
  erythroplastids, 21
  Heinz body, 20
  Howell-Jolly bodies, 20
  hypochromia, 19
  macrocytes, 18
  microcytes, 18
  normal, 16
  nucleated erythrocytes, 20–21
  parasites, 21
  poikilocyte, 19–20
  polychromasia, 18–19
  Rouleaux formations, 18
  spherocytes, 18
Erythrocytes, 3–41, *9*
  accelerated destruction of
    causes for, 31*t*
  anemia: diagnosis and classification, 26–30, 32–40
  antigen and antibody detection, 24–26
  blood reticulocyte evaluation, 21–23
  bone marrow examination, 23–24
  destruction of, 11–13
  erythrokinetics, 7, 9, 11
  erythron, 3
  evaluating, 13–14
  extravascular destruction of, pathway, *12*
  globin synthesis, 4
  heme synthesis, 3
  increased production of, 11
  intravascular destruction of, *12*
  intravascular destruction of, pathway, *12*
  metabolic pathways, *6*
  nucleated, identifying, 59
  oxidants and, 35
  peripheral blood smear, 15–16, 18–21
  polycythemia, 40–41
  red blood cell indices, 14–15
  in urinary sediment, *265*
  in urine sediment examination, 269
Erythroid burst-forming units, *8*
Erythroid colony-forming units, *8*
Erythrokinetics, 7, 9, 11
Erythroleukemia, bone marrow of cat, *85*
Erythroleukemia (m6), 97–98
Erythron, definition and description of, 3
Erythroplastids, description of, 21
Erythropoiesis, 9, 11
  characteristics of, 9
  regulation of, 11
  sequence of, *9*
Erythropoietin (Epo), actions of, 11
Escherichia coli, equine hyperammonemia and, 245
Essential thrombocythemia, 100
  blood of cat, *86*
Essential thrombocytosis, 122–123
Estrogen-induced pancytopenia (case 4), 392–393
  laboratory data, *392–393*
  problems and summary, 393
  signalment, presenting problems, 392
Estrus
  cornified/superficial cells predominant during, *346*
  cytologic characteristics of, 360
ET. *See* Essential thrombocytosis
Ethmoid hematomas, nasal, 358
Ethylene glycol toxicosis, 272, 430–431
  calcium oxalate monohydrate crystals in, 271
Ewes, ketonuria and pregnancy disease of, 261
Excessive ineffective granulopoiesis, 73
Exercise
  acid-base balance, electrolytes and, 155
  hyperkalemia in hypothyroid dogs and, 161
Exocrine pancreatic insufficiency
  diagnosis of, 238–239
  in dogs, 238
Exocrine pancreatic insufficiency, diabetes mellitus, and chronic renal failure (case 15), 421–423
  laboratory data, *421–422*
  problems, 422–423
  signalment, presenting problems, 421
  summary, 423
Exogenous creatinine clearance, 278
Exogenous dyes, uptake and excretion of, hepatic function evaluation and, 212
Exogenous lipids, 184
Extracellular osmolality, 146–147
Extracellular parasites, 21
Extrahepatic biliary disease, clinical features, characteristics, and laboratory findings on, 226

Free ebooks ==> www.Ebook777.com

Extravascular hemolysis
causes of accelerated erythrocyte destruction and, 31*t*
clinical and laboratory characteristics of, 33
examples of aids in identification of, 33
mechanisms of, 30, 32–33
Extrinsic pathway of coagulation, mechanisms and disorders of, 126
Exudates, 347, 350
nasal, 356–358

F
Factor II, 124
Factor V, 108, 111
Factor VII, 124
Factor VIIa, 108
Factor VIII, 108
Factor IX, 124
Factor X, 108, 124
disorders of common pathway of coagulation and, 128
hereditary deficiency of, in dogs and cats, 128
Factor XI, 111
Factor XII (Hageman factor), 124
Factor XIII, 125
False-negatives, 377, 378, 381
False-positives, 377, 378, 381
Familial hyperchylomicronemia, 188
Fasting hyperlipidemia, 187
pancreatic inflammation and necrosis and, 236
Fat droplets, in urinary sediment, *265*, *270*
Fats, mature lipocytes from, *336*
Fatty casts, 270
FDPs. *See* Fibrin(ogen) degradation products
FE. *See* Fractional excretion
Fecal alpha$_1$ PI, 244–245
Fecal proteolytic activity, exocrine pancreatic insufficiency and, 239
Feline basophil, *48*
Feline basophil and small lymphocyte, *47*
Feline erythrocytes
morphology of, 16
platelets and, *17*
Feline hepatic lipidosis, biochemical profile, 228*t*
Feline hyperthyroidism, function tests in diagnosis of, *314*
Feline immunodeficiency virus, neutropenia and, 72
Feline infectious panleukopenia
case 9, 405–407
laboratory data, *405–406*
presenting problems, 405
problems, 406–407
signalment, presenting problems, 405
summary, 407
neutropenia and, 72
Feline infectious peritonitis
case 7, 400–401
laboratory data, *400–401*
presenting problems, 400
problems and summary, 401
effusions caused by, 350
macrophages, neutrophils, and background precipitate from, *344*
Feline leukemia virus (FeLV), 72, 89, 120, 121
Feline lower urinary tract disease (feline urologic syndrome) (case 20), 436–437
laboratory data, *436–437*
problems, 437
signalment, presenting problems, 436
Feline lymphoma, 89–90
anatomic and functional features, 89–90
laboratory features, 90
role of viral agents, 89
Feline mast cell leukemia, poorly differentiated mast cells in, *10*
Feline trypsin-like immunoreactivity, 179, 234
FeLV-induced anemias, 39
FeLV-infected cats, macrocytic erythrocytes in, 15
Ferrets, lymphoma in, 90
Ferritin, defined, 5
Ferrochelatase, 3
Ferroportin 1, 5
FGF. *See* Fibroblast growth factor
Fibrin-bound plasmin, 130
Fibrin formation, *130*
clot retraction evaluation scale, *116*
scheme for, *124*
Fibrinogen, 111
hereditary deficiency of, in goats and dogs, 128
as index of inflammation in animals, 179
Fibrinogen concentration, 136, 177
Fibrin(ogen) degradation products, 137
Fibrinolysis, 107
avian, 132
formation of, *130*
laboratory evaluation of, 132–138
mechanisms and disorders of, 129–131
Fibrinolytic factors, assays for, 138
Fibroblast growth factor, 111
Fibroblast growth factor-4, 110
Fibrosarcomas, nasal, 357
Filarids, 21
Fine-needle aspiration, 331–332
FIP virus, platelet function and, 121
Fitzgerald factor, 126
Fletcher factor, 126
Flow cytometry
assays, 116–117
platelet counts and, 112
Fluid collection, 332
FN. *See* False-negatives
FNA. *See* Fine-needle aspiration
Folate, malabsorption and, 240
Folate concentration, malassimilation diseases and typical laboratory changes in, 241*t*
Folate measurement, exocrine pancreatic insufficiency and, 239
Foxhounds, Pelger-Huet anomaly in, 60
FP. *See* False-positives
fPLI, 237
Fractional excretion, 279
Free hemoglobin, 12
Free T4 dialysis, confirming hyperthyroidism and, 315
Fructosamine, in glycated protein assays, 196
fT4. *See* Serum-free T4
fTLI. *See* Feline trypsin-like immunoreactivity

www.Ebook777.com

Fungal hyphae, *335*
in urinary sediment, *266*
Fungi
pulmonary lesions and, 359
in urine, 271
Fusocytes, description of, 19

G
GALT. *See* Gut-associated lymphoid tissue
Gamma-globulins, 174, *174*, 175
Gamma glutamyl transferase, 216–217, 264
Gastritis, serum lipase activity and, 233
Gastrointestinal disease, hypocalcemia and, 301
Gastrointestinal tract, urea excretion via, 275
GDH. *See* Glutamate dehydrogenase
Geese, urine transparency in, 255
Gentamycin, renal tubular damage and high dosages of, 260
German Shepherds
Aspergillus terreus in, 271
exocrine pancreatic insufficiency in, 238
megaesophagus and, 243
small intestinal bacterial overgrowth in, 240
GFR
measurement of, 278–279
endogenous creatinine clearance, 278
exogenous creatinine and isohexol clearance, 278–279
GGT. *See* Gamma glutamyl transferase
GGT activity, increased, urea toxicosis and, 460
Giant platelets, 113
Giant Schnauzers
cobalamin deficiency in, 241
macrocytic anemia in, 14
Giardiasis, testing for, malabsorption and, 240
Glanzmann thrombasthenia, 118, 119
Glitter cells, defined, 269
Globin, 3
synthesis of, 4
Globulin concentrations, abnormalities in, 223–224
Globulins
electrophoresis and separation of, 174–175
measuring, 176–177
Glomerular filtration rate, prerenal azotemia and, 275
Glomerulotubular imbalance, renal amyloidosis and, 435
Glomerulus
glucose filtered by, 259
ketones filtered by, 260
Glucagon
gluconeogenesis and, 192
glycogenolysis and, 191–192
metabolic effects of, 316
Glucocorticoids, hyperglycemia and, 192
Glucocorticoid secretion, adrenal cortex and, 317
Glucocorticoid suppression of thyroid function (case 32)
endocrine laboratory data, *466*
signalment, medical history, presenting problems, physical findings, 466
Glucocorticoid treatment, corticosteroid hepatopathy and, 226
Gluconeogenesis
defined, 192
inadequate, 193
Glucorticoids, decrease in glycogenolysis and, 192
Glucose
excessive use of, 192
fate of blood glucose, 192–193
feline hyperthyroidism and, 314
glucose metabolism in birds, 193
hyperglycemic disorders, 196
hypoglycemic disorders, 196
maintenance of blood glucose concentration, 193
means of evaluating glucose metabolism, 193–196
sources of blood glucose, 191–192
Glucose control, Levey-Jennings chart, *368*
Glucose homeostasis
abnormalities in, 223
hepatic function evaluation and, 212
Glucose intake, reduced, 193
Glucose in urine, 259–260
interpretation of positive test result, 260
measurement of, 260
physiology, 259–260
Glucose metabolism
in birds, 193
disorders of, 190–191
evaluating, 193
Glucose tolerance tests, 194–195
diabetes mellitus and, 316
feline hyperthyroidism and, 314
intravenous or oral, 195
Glucosuria, 194
right abomasal displacement and, 444
urea toxicosis and, 461
in various animals, 260
Glutamate dehydrogenase, hepatocellular leakage enzymes and, 214
Glutaraldehyde coagulation test, for detecting IgG, 177
Glycated proteins, 196
Glycoalbumin, in glycated protein assays, 196
Glycogen, reduced hepatic storage of, 192
Glycogenesis, 192
Glycogenolysis, promotion of, 191–192
Glycogen storage, hepatic function evaluation and, 212
GM-CSF, 110
Golden Retrievers, megaesophagus and, 243
Gold standard, 376, 377
Gout, avian, 353
Gradual water deprivation test, 274
Granular casts, in urine sediment examination, 270
Granule release, platelet aggregation and, 110–111
Granules, types of, 45
Granulocyte colony-forming units, *8*
Granulocyte-CSF, 49
Granulocyte/macrophage-CSF, 49
Granulocyte/monocyte colony-forming units, *8*
Granulomatous disease, hypercalcemia in dogs and, 301
Granulopoiesis
excessive ineffective, 73
regulation of, 49–50
sequence of, *49*
Granulopoietic hyperplasia, neutropenia and, 73
Grass tetany, hypomagnesemia in cattle and, 304
Grey Collies, cyclic hematopoiesis in, 73, 119

Free ebooks ==> www.Ebook777.com

Greyhounds
alpha-globulin peaks on serum electrophoretogram and, 180
basal serum T4 and fT4 concentrations in, 309
higher MCV in, 14
Growth hormone
gluconeogenesis and, 192
hyperglycemia and, 192
GT. *See* Glanzmann thrombasthenia
Gut-associated lymphoid tissue, 57, 58

H
HALT. *See* Head-associated lymphoid tissue
Haptoglobin, 12, 180
Haptoglobin-hemoglobin binding assay, 177
Hb concentration
erythrocyte evaluation and, 13–14
factors affecting, 14
Hct. *See* Hematocrit
HDLs. *See* High-density lipoproteins
Head-associated lymphoid tissue, 57
Heart failure, chylothorax in cats and, 350
Heartworm disease, platelet function and, 121
Heartworm infestation, hyperadrenocorticism and diabetes mellitus, with other complications and, 470
Heinz bodies
description of, 20
in erythrocyte ghosts, acetaminophen toxicosis in cat, *17*
formation of, erythrocytes and, 35
red maple toxicosis, horse, *17*
Hemacolor, 16
Hemangiopericytomas, 341
plump spindle cells from, *336*
Hemarthrosis, 354
rodenticide toxicosis and, 449
Hematocrit
anemia detection with, 27
defined, 13
factors affecting, 14
hemorrhage and, 29
Hematology reference intervals, 372*t*
Hematomas, 339–340
Hematopoiesis, *8*
Hematopoietic cells, in bone marrow, *10*
Hematopoietic neoplasia, 83–101
acute lymphocytic leukemia, 91–92
chronic lymphocytic leukemia, 92–93
large granular lymphocyte lymphoma/leukemia, 93
lymphoma, 83, 87–91
lymphoproliferative disorders, 83
myeloproliferative disorders, 95–101
acute myeloid leukemia, 96–98
chronic myeloid leukemia, 99–100
general characteristics, 96
mast cell leukemia, 100
myelodysplastic syndrome, 98
myeloid-derived macrophage and dendritic cell neoplasms, 100–101
plasma cell tumors, 93–95
Hematopoietic stem cells, neutropenia and, 72
Hematuria
occult blood test and, 262
rodenticide toxicosis and, 449
urine color and, 254
von Willebrand's disease and, 457
Heme, synthesis of, 3
Hemochromatosis, in Salers cattle, 4
Hemocytometer, 14, 58
manual platelet counts with, 112
Hemoglobin
increased, artifacts of lipemia, hemolysis, and improper sample handling and, 473
occult blood test and, 261, 262
transport of, 3
Hemoglobin Alc, in glycated protein assays, 196
Hemoglobinemia, intravascular hemolysis and, 35
Hemoglobinemic plasma, color of, 13
Hemoglobinopathies, 4
Hemoglobinuria, 288
occult blood test and, 262
Hemolysis, 4
Hemolytic anemia
acute intravascular, 389–391
characteristics of, 30
clinical findings for, 30
differentiating causes of, 30
laboratory findings for, 30
serum iron and, 4
Hemolytic tests, 25
Hemopexin, 12
Hemophagocytic histocytic sarcoma, characteristics of, 101
Hemophilia B, 127
Hemoproteus spp., 21
in cytoplasm of avian erythrocyte, *63*
Hemorrhage
acute *vs.* chronic, causes of, 28*t*
clinical findings, 28
external and internal, differential features of anemia with, 29–30
hypocalcemia and, 301
laboratory findings, 29
Hemorrhage into urinary tract, urogenital proteinuria and, 259
Hemorrhagic anemia (case 1)
laboratory data, *385*
signalment, presenting problems, problems and summary, 385
Hemorrhagic cystitis, feline lower urinary tract disease and, 437
Hemorrhagic effusions, 351
Hemosiderin, 5
Hemosiderinuria, 35
Hemostasis, 107–139
antithrombotic properties of endothelial cells, 107–108
coagulation
laboratory evaluation of, 132–138
mechanisms/disorders of vitamin-K-dependent factors, 129
coagulation and fibrinolysis, 123–139
mechanisms/disorders of common pathway of coagulation, 128–129

www.Ebook777.com

Hemostasis (*continued*)
mechanisms/disorders of extrinsic pathway of coagulation, 126
mechanisms/disorders of intrinsic pathway of coagulation, 126–128
mechanisms of coagulation, 123–126
defined, 107
endothelium, 107
essential concepts, 107
fibrinolysis
avian coagulation and, 132
laboratory evaluation of, 132–138
mechanisms/disorders of, 129–131
hepatic synthesis and, 224
platelets, 108–123
disorders of, 117–123
function, 110–111
laboratory evaluation of, 111–117
morphology, 108–109
production, 109–110
procoagulant properties that follow stimulation of endothelial cells, 108
Hemostasis disorders, patterns for, 133*t*
Hemostasis reference intervals, 373*t*
Henderson-Hasselbalch equation, 149
Heparan sulfate, 108
Hepatic abnormalities, laboratory tests and detection of, 211
Hepatic atrophy, 211
Hepatic diseases
biochemical profiles of, 224–228
acute submassive or massive hepatic necrosis, 225
centrilobular hepatic lesions caused by hypoxia, 224–225
cholangitis, cholangiohepatitis, and extrahepatic biliary disease, 226
chronic, progressive liver disease, 226–227
congenital portosystemic shunts, 227
corticosteroid hepatopathy, 226
hepatic lipidosis, 225–226
large focal hepatic lesions, 225
multifocal hepatic necrosis, 224
primary or metastatic hepatic neoplasia, 227–228
hyperthyroidism and, 455
secondary hyperlipidemia and, 189
Hepatic disorders
biochemical profiles of
typical changes in animals, 228*t*
serum lipase activity and, 233
Hepatic encephalopathy (hepatic fibrosis) (case 12), 413–414
laboratory data, *413–414*
problems and summary, 414
signalment, presenting problems, 413
Hepatic enzyme alterations, 213–217
hepatocellular leakage enzymes, 213–214
induced hepatic enzymes, 214–217
Hepatic functions, laboratory tests and evaluation of, 212
Hepatic insufficiency, 211–212
defined, 211
mechanisms of, 211
Hepatic laboratory tests, 212–217
alterations in hepatic synthesis and homeostasis, 223–224
hepatic enzyme alterations, 213–217
hepatic uptake, conjugation, and secretion, 217–219, 221–223
Hepatic lipidosis
clinical features and characteristics of, 225
laboratory findings on, 225–226
Hepatic necrosis, acute submassive or massive, clinical features, characteristics, and laboratory findings on, 225
Hepatic neoplasia, primary or metastatic, clinical features and laboratory findings on, 227–228
Hepatic photosensitivity, 223
Hepatocellular disease, exocrine pancreatic insufficiency, diabetes mellitus, chornic renal failure and, 423
Hepatocellular injury
acute pancreatic necrosis and, 419
chronic hemorrhagic anemia and, 395
conditions related to, 211
Hepatocellular leakage enzymes, 213–214
alanine aminotransferase, 213–214
aspartate aminotransferase, 214
defined, 213
general characteristics, 213
glutamate dehydrogenase, 214
lactate dehydrogenase, 214
sorbitol dehydrogenase, 214
Hepatocellular leakage/necrosis, 211
Hepatotoxicity, hepatic lipidosis and, 225
Hepatozoon canis gametocyte, in canine monocyte, *63*
Hepatozoon spp., in leukocytes, 61
Hepcidin, iron homeostasis and, 5
Hephaestin, iron transport and, 5
Hereditary, sex-linked, factor IX deficiency or hemophilia, 127
Hereditary, sex-linked, factor VIII: coagulant, 127
Hereditary deficiency of prekallikrein, 127
Hereditary factor XI deficiency, 127
Herpesvirus, heteropenia in birds and, 72
Heteropenia in birds
causes of, 72*t*
excessive demand for or destruction of heterophils in birds, 71
excessive ineffective granulopoiesis, 73
margination of circulating neutrophils, 71
reduced production of heterophils in birds, 71–74
Heterophilia in birds, 64–71
causes of, 65*t*
corticosteroid-induced, 66–68
inflammation and infection, 68–71
clinical findings, 68–69
leukogram changes, 68
mechanisms, 69–70
species characteristics, 70–71
leukogram findings, 64
Heterophil-lymphocyte reversal, in avian species, 71
Heterophils, 45
avian, 45, *46*
avian, morphology, 49
toxic, 60

Free ebooks ==> www.Ebook777.com

High-density lipoproteins, 185
High-dose dexamethasone suppression test, protocol and interpretation of, 321
High-dry magnification, 16
Histiocytic sarcoma cells, characteristics of, 101
Histiocytic sarcoma of interstitial dendritic cell origin, 101
Histiocytomas, *337*, 342
Histoplasma capsulatum yeasts, *334*, 338
Horses
  acute intravascular hemolytic anemia in, 389–391
  acute salmonellosis in, 397–399
  bile acid measurements for, 221
  continuous glucose monitoring devices used for, 194
  corticosteroid-induced neutrophilia in mammals/heterophilia in birds and, 68
  hepatic encephalopathy in, 413–414
  hyperammonemia in, 245–246
  hypercalcemia and renal disease in, 300
  lymphoma in, 90–91
  muscle disease, myoglobinuric nephrosis in, 427–429
  neutrophilia and heterophilia of inflammation/infection in, 70–71
  performing TRH stimulation test in, 315
  polycythemia vera in, 100
Horse urine, cloudiness of, 254
Howell-Jolly bodies
  description of, 20
  dog, *17*
Hyaline casts, 270
  in urinary sediment, *266*
Hydrogen ion concentration, 148
Hydrogen ion concentration (pH) in urine, methods of measurement and interpretation of, 263
Hyperadrenocorticism, 318
  case 26, 450–452
    laboratory data, *450–451*
    problems, 451–452
    signalment, medical history, presenting problems, 450
    summary, 452
  corticosteroid hepatopathy and, 226
  other laboratory abnormalities in, 322–323
Hyperadrenocorticism and diabetes mellitus, with other problems (case 34), 468–471
  laboratory data, *468–469*
  problems, 469–471
  signalment, presenting problems, 468
  summary, 471
Hyperalbuminemia, 178
  acute pancreatic necrosis and, 420
  artifacts of lipemia, hemolysis, and improper sample handling and, 473
  feline infectious panleukopenia and, 407
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  nephrosis and perirenal hemorrhage and, 441
Hyperammonemia, 221
  equine, 245–246
  hepatic disease and, 414
  hepatic insufficiency and, 222
  portosystemic venous shunting and, 222
  urea toxicosis and, 461
Hyperamylasemia
  causes of, 232*t*
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  poor specificity for pancreatitis, 233
Hyperbilirubinemia, 35
  causes of, 218
  hepatic disease and, 414
  hepatic failure and, 417
  pancreatic inflammation and necrosis and, 236
Hypercalcemia
  artifacts of lipemia, hemolysis, and improper sample handling and, 474
  canine adrenal insufficiency and, 300
  canine lymphoma and, 89
  causes of, 299–300, 301*t*
  end stage renal disease with uremic pneumonitis and, 464
  multicentric lymphoma and, 409
Hypercalcitonism, in bulls with thyroid C-cell neoplasms, 302
Hypercapnia, 168*t*
  hypoventilatory diseases and circumstances associated with, 168*t*
Hyperchloridemia, urea toxicosis and, 460
Hypercholesterolemia
  hypothyroidism and, 313
Hypercholestrolemia, renal amyloidosis and, 435
Hypercoagulation, factors related to, 131
Hypereosinophilic syndrome, 74
Hyperfibrinogenemia, 178
  acute intravascular hemolytic anemia and, 390
  acute salmonellosis and, 398
  disseminated intravascular coagulation and, 412
  hepatic disease and, 414
  muscle disease, myoglobinuric nephrosis and, 428
  nephrosis and perirenal hemorrhage and, 441
  right abomasal displacement and, 444
Hyperglobilinemia, artifacts of lipemia, hemolysis, and improper sample handling and, 473
Hyperglobulinemia, 178–181
  A/G ratio decreased in, 181
  disseminated intravascular coagulation and, 412
  plasma cell myeloma and, 94
  urea toxicosis and, 460
Hyperglycemia, 190
  acute renal failure (ethylene glycol toxicosis) and, 431–432
  acute septic mastitis and, 438
  causes of, 197*t*
  feline lower urinary tract disease and, 437
  glucosuria and, 260
  hyperthyroidism and, 455
  right abomasal displacement and, 444
Hyperglycemia with glucosuria
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  mild, acute pancreatic necrosis and, 420
  nephrosis and perirenal hemorrhage and, 441–442
Hyperglycemia without glucosuria, hyperadrenocorticism and, 451

www.Ebook777.com

Hyperglycemic disorders, 196
Hyperinsulinism, 315
  detecting, 194
  laboratory assessment of endocrine pancreas and, 317
Hyperkalemia, 279, 289
  acute renal failure (ethylene glycol toxicosis) and, 432
  adrenal insufficiency and, 323
  artifacts of lipemia, hemolysis, and improper sample handling and, 474
  end stage renal disease with uremic pneumonitis and, 464
  feline lower urinary tract disease and, 437
  mechanisms and causes of, 159, 160*t*, 161
Hyperlactatemia, causes of, 200
Hyperlipasemia, 280
  causes of, 232*t*
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  interpretation of, 233
Hyperlipidemia
  causes of, 188*t*
  defined, 187
  fasting, pancreatic inflammation and necrosis and, 236
Hyperlipoproteinemia, 187
Hypermagnesemia, 280
  causes of, 304
Hypermylasemia, 280
Hypernatremia, 157*t*, 158
Hypernatremic hypovolemia, urea toxicosis and, 460
Hyperosmolality, 147
  hyperadrenocorticism and diabetes mellitus with, 468–471
Hyperosmolality of ECF, urea toxicosis and, 460
Hyperparathyroidism, primary, *296*
Hyperphosphatemia, 279
  causes of, 303, 303*t*
  end stage renal disease with uremic pneumonitis and, 464
  feline lower urinary tract disease and, 437
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  nephrosis and perirenal hemorrhage and, 442
  renal amyloidosis and, 435
  renal failure and, 423
Hyperplastic/reactive lymph node, 347
Hyperproteinemia, 177–181
  acute intravascular hemolytic anemia and, 390
  acute pancreatic necrosis and, 420
  acute renal failure (ethylene glycol toxicosis) and, 431
  acute salmonellosis and, 398
  artifacts of lipemia, hemolysis, and improper sample handling and, 473
  feline infectious panleukopenia and, 406
  feline infectious peritonitis and, 401
  hyperadrenocorticism and diabetes mellitus, with other complications and, 471
  with hyperglobulinemia and monoclonal gammopathy, plasma cell myeloma and, 94
  nephrosis and perirenal hemorrhage and, 441
  pancreatic inflammation and necrosis and, 236
  pyometra and, 404
  relative, 177–178
  urea toxicosis and, 459, 460
Hypersplenism, 33
Hyperthyroidism, 313–315
  case 27, 453–455
    laboratory data, *453–454*
    problems, 454–455
    signalment, presenting problems, 453
    summary, 455
  in cats, 313
  confirming, additional tests for, 315
  diagnosis of, 315
  feline, function tests in diagnosis of, *314*
  hepatic lipidosis and, 225
  hyperthyroidism and, 455
  laboratory abnormalities in, 313–314
Hypertriglyceridemia, 186
  pancreatic inflammation and necrosis and, 236
Hyperuricemia, renal disease in birds and, 278
Hyperventilation, causes of, 169*t*
Hypervitaminosis D, hypercalcemia and, 300
Hypoadrenocorticism, 318–319
  megaesophagus and, 243–244
Hypoalbuminemia, 181
  chronic liver failure or atrophy and, 224
  hepatic failure and, 416
  inflammatory bowel disease and, 425
  rodenticide toxicosis and, 448
Hypoaldosteronism, hyperkalemia and, 159
Hypocalcemia, 298
  acute pancreatic necrosis and, 420
  acute renal failure (ethylene glycol toxicosis) and, 432
  causes of, 301–302, 301*t*
  hepatic failure and, 417
  inflammatory bowel disease and, 426
  nephrosis and perirenal hemorrhage and, 442
  parturient paresis in cattle and, 302
  protein-losing enteropathy and, 245
  renal amyloidosis and, 435
  renal failure and, 423
Hypocapnia, 148, 169, 169*t*
Hypocarbia, 148
Hypochloridemia
  acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
  acute renal failure (ethylene glycol toxicosis) and, 432
  pyometra and, 404
  renal disease and, 280
  right abomasal displacement and, 444
Hypocholesterolemia, hepatic failure and, 416
Hypochromasia, iron deficiency in dog, *17*
Hypochromia, 19
Hypocoagulation, factors related to, 131
Hypocobalaminemia, 241
Hypofibrinogenemia, 182
Hypoglobulinemia, 182
Hypoglycemia, 190
  artifacts of lipemia, hemolysis, and improper sample handling and, 474
  causes of, 198*t*
  end stage renal disease with uremic pneumonitis and, 464
  hepatic failure and, 416

Free ebooks ==> www.Ebook777.com

inflammatory bowel disease and, 426
pyometra and, 404
Hypoglycemic disorders, 196
Hypokalemia, 280, 289
acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
mechanisms and causes of, 160*t*, 161–162
nephrosis and perirenal hemorrhage and, 442
right abomasal displacement and, 444
Hypomagnesemia, causes of, 304
Hyponatremia, 158
acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
adrenal insufficiency and, 323
renal disease and, 280
Hyponatremic dehydration
acute salmonellosis and, 398
nephrosis and perirenal hemorrhage and, 442
pyometra and, 404
Hypo-osmolality, 147
Hypoparathyroidism
hypocalcemia and, 301
primary, *296*
Hypophosphatemia, 300
artifacts of lipemia, hemolysis, and improper sample handling and, 474
causes of, 303–304, 303*t*
hemolysis and, 35
Hypoproteinemia, 181–182
causes of, 244
end stage renal disease with uremic pneumonitis and, 464
hepatic failure and, 416
inflammatory bowel disease and, 425
relative, 181
renal amyloidosis and, 435
rodenticide toxicosis and, 448
serum iron and, 4
Hyporesponsive platelets, 120
Hyposthenuria
defined, 257
hepatic failure and, 417
Hypothalamic-pituitary-adrenal axis, adrenal function tests and, *319*
Hypothalamic-pituitary-thyroid axis, in euthyroid animals, overview of, *305*
Hypothyroidism, 312–313
anti-thyroglobulin autoantibodies in dogs with, 309
in dogs, anti-T4 autoantibodies and, 310
early primary (case 31)
endocrine laboratory data, *465*
signalment, medical history, presenting problems, physical findings, comment, 465
incidence of, 312
laboratory abnormalities in, 313
megaesophagus and, 243
primary (and phenobarbital administration) (case 33)
endocrine laboratory data, *467*
signalment, medical history, presenting problems, physical findings, 467
secondary, 313
secondary hyperlipidemia and, 188
serum iron and, 4
sulfonamide administration and drug-induced form of, 307
thyroid function testing in birds, 312
Hypoventilatory diseases, hypoxemia, hypercapnia, and respiratory acidosis, 168*t*
Hypoventilatory respiratory disease, end stage renal disease with uremic pneumonitis and, 463
Hypovolemia, 146
Hypoxemia, 168*t*
acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
hypoventilatory diseases and circumstances associated with, 168*t*
Hypoxia
biochemical profile, 228*t*
centrilobular hepatic lesions caused by, 224–225

I
IALP. *See* Intestinal and placental ALP isoenzyme
ICG. *See* Indocyanine green
Icteric plasma, color of, 13
IDEXX QBC VetAutoReader, 111
IDEXX SNAP cPL, 234
Idiopathic hyperlipodemia, 187
IDLs. *See* Intermediate-density lipoproteins
IL-3, 7, 11, 110
IL-9, 7
IL-11, 7, 110
IL-12, 110
IL-1-alpha, 110
Immune-mediated hemolytic anemias, causes of, 32
Immune-mediated thrombocytopenia, 122
Immunoblot, 177
Immunocytes, 60
Immunoglobulin, deficiency in, diseases characterized by, 182
Immunonephelometry, 177
Inappetence, hypertriglyceridemia and, 189
In-clinic testing
advantage with, 368
customer service and, 369
Indirect antiglobulin test, 25
Indirect-reading bilirubin, 218
Indocyanine green, 223
Induced hepatic enzymes, 214–217
alkaline phosphatase, 214–215
bone ALP isoenzyme, 216
corticosteroid ALP isoenzyme, 215–216
gamma glutamyl transferase, 216–217
general characteristics of, 214
intestinal and placental ALP isoenzyme, 216
Infection, left shift as hallmark of, 68, 69
Inflammation
left shift as hallmark of, 68, 69
platelets and, 111
Inflammatory bowel disease, hepatic lipidosis and, 225
Inflammatory bowel disease (malabsorption with protein-losing enteropathy) (case 16), 424–426
laboratory data, *424–425*
problems, 425–426
signalment, presenting problems, 424
summary, 426

www.Ebook777.com

Inflammatory lesions, 333
infectious agents seen in, 338
Inflammatory leukogram
acute pancreatic necrosis and, 419
acute renal failure (ethylene glycol toxicosis) and, 432
hepatic disease and, 414
renal abscess and secondary disseminated intravascular coagulation and, 411
Inflammatory response, functions of other mediators of, 50
Inherited lipoprotein lipase deficiency, 188
Injection site reactions, 333
with intracellular and extracellular globular foreign material, *334*
Institutional laboratories, quality control in, 367–368
Insulin
cellular uptake of glucose and, 192
metabolic effects of, 315–316
Insulin antagonists, 193
Insulin-dependent hypertriglyceridemia, 188
Insulin/glucose ratios, amended, 194
Interleukins, lymphocyte-derived, function of, 58
Intermediate-density lipoproteins, 185
Intestinal and placental ALP isoenzyme, 216
Intracellular parasites, 21
Intravascular hemolysis, 30
causes of accelerated erythrocyte destruction and, 31*t*
hemoglobinemia and, 35
mechanisms of, 34–35
complement-mediated lysis, 34
membrane alterations by other mechanisms, 35
osmotic lysis, 34–35
oxidative injury, 34
physical injury, 34
Intravascular hemolytic anemia, clinical and laboratory characteristics of, 35
Intravenous glucose tolerance test, 195
Intrinsic pathway of coagulation, mechanisms and disorders of, 126–128
Ionized calcium, 297–298
Ion-selective electrodes, calcium measurement and, 298
Iron, storage of, 5
Iron deficiency anemia
laboratory findings in, 39–40
microcytic, hypochromic anemia with variable neutrophil and platelet counts and, 39
Iron-lack anemia, 394–396
Iron metabolism
TIBC and, 4
transferrin and, 5
transport of iron, 4
Iron overload, 4
Islets of Langerhans, 315
Isohexol clearance, 278
Isosthenuria, 257
end stage renal disease with uremic pneumonitis and, 464
renal failure and, 423

J
Joint fluid, *344*

K
Keratin-producing cyst or tumor, *335*
Keratin-producing lesions, 339
Keratocytes
description of, 19
dog, *17*
Ketoacidosis, 199
hyperadrenocorticism and diabetes mellitus with, 468–471
low blood insulin concentrations and, 316
Ketone bodies, 197
Ketonemia
causes of, 199
low blood insulin concentrations and, 316
Ketones, 186, 191, 197, 199
measurement of, 199
production of, 199
in urine, 260–261
interpretation of positive test result, 260–261
measurement of, 260
physiology, 260
Ketonuria, 260–261
causes of, 199
low blood insulin concentrations and, 316
Ketosis, 260
acute septic mastitis, increased anion gap and, 438
Kidneys
serum phosphorus regulated by, 299
urea excretion and, 275
Klebsiella, equine hyperammonemia and, 245

L
Laboratory tests
overlapping values for, *376*
theoretically perfect, histogram of, *376*
Labrador Retrievers
acid-base balance and electrolytes in, 155
glucosuria in, 260
inherited vitamin-K-dependent multifactor coagulopathies in, 129
Lactate, 191, 199–200
measurement of, 200
muscle and, 289
production of, 199
Lactate dehydrogenase
diagnostic significance of, 285, 287
diseases of muscle origin and, 286*t*
hepatocellular leakage enzymes and, 214
muscle and isoenzymes of, 284–285
LALP. *See* Liver ALP isoenzyme
Landseer-ECT thrombopathia, 118
Large focal hepatic lesions, clinical features, characteristics, and laboratory findings on, 225
Large granular lymphocyte (LGL) lymphoma/leukemia
definition and occurrence, 93
differential diagnoses, 93
laboratory features, 93
Large granular lymphoma
in cats, 89
intestine, cat, *84*
Latex agglutination, 177

Free ebooks ==> www.Ebook777.com

Latex agglutination test, for detecting IgG, 177
Laurell rocket technique, Protein C and, 137
LCFAs. *See* Long-chain fatty acids
LDH. *See* Lactate dehydrogenase
  canine lymphoma and, 287
  skeletal muscle disease in birds and, 287
LDLs. *See* Low-density lipoproteins
Lead, inhibition of heme synthesis and, 3
Lead toxicosis, urine color and, 254
Left shift, neutrophilia and heterophilia of inflammation and, 68, 69
Leishmania spp., 53, 339
Leptin, obesity and, 190
Leptocytes
  description of, 19
  dog, *17*
Leukemia inhibitory factor, 110
Leukemias
  anemia, maturation abnormalities and, 37
  defined, 87
  morphologic appearance of forms of, *84–86*
  platelet defects and, 120
Leukemic cells, in bone marrow, *10*
Leukemoid response, 70
Leukocyte adhesion deficiency type III disorder, 119
Leukocyte count, differential, 59
Leukocyte esterase activity, in urine, 263
Leukocytes, 45–80
  basophils, 54–55
  bone marrow examination, 64–80
    basopenia, 77
    basophilia, 76–77, 76*t*
    eosinopenia, 75–76
    eosinophilia, 74, 75*t*
    lymphocytosis, 77–78, 77*t*
    lymphopenia, 78, 79*t*
    monocytopenia, 74
    monocytosis, 74
    neutropenia in mammals and heteropenia in birds, 71, 72*t* 72–73
    neutrophilia in mammals and heterophilia in birds, 64, 65–71, 65*t*
    prognosis and responses of, 79–80
  eosinophils, 54
  infectious organisms within cytoplasm of, *62–63*
  laboratory evaluation of, 58–64
    blood smear evaluation, 59–61, 64
    white blood cell count, 58–59
  lymphocytes, 56–58
  mast cells, 55–56
  monocytes-macrophages, 52–53
  morphology
    function, production, and kinetics, 45
    in health and disease, *46–48*
  neutrophils and avian heterophils, 45, 48–52
  organisms or diagnostic inclusions observed in, 61
  prognosis and responses of, 79–80
    favorable prognosis, 80
    general considerations, 79–80
    guarded and poor prognosis, 80
  relative number of, 16
  in urinary sediment, *265*, *266*, *267*, 269
Leukocytosis
  end stage renal disease with uremic pneumonitis and, 463
  feline infectious peritonitis and, 401
  hyperadrenocorticism and, 322, 451
  hyperadrenocorticism and diabetes mellitus, with other complications and, 470
  hyperthyroidism and, 454
  muscle disease, myoglobinuric nephrosis and, 428
  pyometra and, 403
  rodenticide toxicosis and, 448
  urea toxicosis and, 459
  in young healthy horses, 66
Leukocytozoon spp., 21
Leukograms
  interpretation of, 59
  pancreatic inflammation and necrosis and, 235
Leukopenia
  acute salmonellosis and, 398
  feline infectious panleukopenia and, 406
Levey-Jennings charts, 367, *368*
LIF. *See* Leukemia inhibitory factor
Likelihood ratios, 380
Lipase, 233–234
  characteristics of, 233
  measurement of, 231–232
Lipase activity, decreased, artifacts of lipemia, hemolysis, and improper sample handling and, 474
Lipemia
  defined, 189
  diabetes mellitus and, 317
  effects of, on laboratory tests and management of, 189–190
  glucose measurement and, 194
Lipemia-refrigeration test, 186
Lipemic plasma, color of, 13
Lipid metabolism, overview of, *184*
Lipids, digestion of, 184
Lipomas, 341
  mature lipocytes from, *336*
Lipoprotein lipase determination, 187
Lipoproteins
  determination of, 186–187
  synthesis of, 184
Liver, 211–228
  biochemical profiles of hepatic diseases, 224–228
  cholestasis, 212
  decreased hepatic functional mass, 211–212
  hepatic laboratory tests, 212–224
  hepatocellular leakage/necrosis, 211
  portal blood flow alteration, 212
  synthesis/homeostasis of serum constituents and, 223
  vitamin-K-dependent clotting factors produced by, 224
Liver ALP isoenzyme, 215
Liver disease
  chronic, progressive
    biochemical profile, 228*t*
    clinical features and characteristics, 226–227
    laboratory findings on, 227
  hyporesponsive platelets and, 120

www.Ebook777.com

Liver function testing, malabsorption and, 240
L-lactate dehydrogenase, 200
Llamas, elliptical erythrocytes in, 40
Long-chain fatty acids, 184, 185
Loop of Henle, urine volume and, 255
Low-density lipoproteins, 185
Low-dose dexamethasone suppression test
  interpretation of, 321
  protocol for, 320
Lower limit of detection, test validity and, 366
Lowry and modified Lowry methods, for total protein measurement, 175
Lowry (Folin-Ciocalteau phenol) method, for total protein measurement, 175
Low urine specific gravity, pyometra and, 404
Lymphadenitis, 347
Lymphangiectasia, 242–243
Lymph node aspirates, *343*
Lymph node hyperplasia, feline infectious panleukopenia and, 407
Lymph nodes
  hyperplastic/reactive, 347
  normal, 342, *343*, 347
Lymphoblasts, 57, 61
Lymphocytes, 56–58
  cytology, body cavity effusions, 349
  distribution and circulation, 57–58
  function, 58
  life span of, 57
  mature, 56
  morphology, 56–57
  plasma cells and, 56
  production, 57
  reactive, 56
  relative, 60
  small, in chylothorax, *343*
Lymphocytic leukemia, 87
Lymphocytosis
  causes of, 77*t*
  description and mechanisms of, 77–78
Lymphoid neoplasia forms, 83, 87–95
  acute lymphocytic leukemia, 91–92
  chronic lymphocytic leukemia, 92–93
  large granular lymphocyte lymphoma, 93
  lymphoma, 83, 87–91
  plasma cell tumors, 93–95
Lymphoid neoplasms, origination of, 83
Lymphomas, 83, 87–91, 342, 347
  avian, anatomic and functional features/laboratory features, 90
  bovine, 91
  canine, 88–89
    anatomic and functional features, 88–89
    laboratory features, 89
  in cattle, high serum LDH activity and, 287
  clinical staging of, 88
  definitions and comments, 83, 87
  in dogs, *84*
  in dogs, LDH activity and, 287
  equine
    anatomic and functional features, 90
    laboratory features, 91
  feline, 89–90
    anatomic and functional features, 89–90
    laboratory features, 90
    role of viral agents, 89
  hypercalcemia and, 300
  morphologic appearance of forms of, *84–86*
  mustelid, anatomic and functional features/laboratory features, 90
  of nasal cavity, 357
  subclassification, 87–88
    anatomic distribution, 87
    CD expression (immunophenotype), 88
    histologic pattern in lymph nodes, 87
    morphology of neoplastic lymphocytes, 87–88
Lymphopenia
  causes of, 79*t*
  corticosteroid or "stress" leukogram and, 236
  description and mechanisms of, 78
  end stage renal disease with uremic pneumonitis and, 463
  feline infectious panleukopenia and, 406
  hyperadrenocorticism and, 451
  hyperthyroidism and, 454
  inflammatory bowel disease and, 426
  with lymphangiectasia, 243
  neutrophilia and heterophilia of inflammation and, 68
Lymphopoiesis, 57
Lymphosarcoma, 83, *337*
Lysosomal storage diseases, characteristics of, 64
Lysosomes, 109

M
Macroamylase, serum amylase measurement and, 233
Macrocytes, description of, 18
Macrocytic, normochromic anemia with variable neutrophil and platelet counts, causes of, 40
Macrocytosis
  in dogs with portosystemic venous shunts, 14
  hyperadrenocorticism and diabetes mellitus, with other complications and, 469
Macrophages, 333
  activation of, 53
  alveolar, *345*
  cytology, body cavity effusions, 349
  with hemosiderin and hematoidin crystals due to chronic hemorrhage, *344*
  monocyte-derived, 53
  morphology, 52–53
  from sialocele, *336*
Macroplatelet, shift platelet, dog, *17*
Macrothrombocytopenia, in Cavalier King Charles Spaniels, 119
Magnesium
  laboratory abnormalities in, 299–304
  measurement and monitoring of, 299
Malabsorption
  inflammatory bowel disease and, 425
  laboratory detection of malassimilation due to, 239–243
  small intestinal bacterial overgrowth and, 240–242

Free ebooks ==> www.Ebook777.com

Malassezia spp. yeasts, 338
footprint-shaped, *334*
Malassimilation
laboratory detection of, due to malabsorption, 239–243
lymphangiectasia, 242–243
small intestinal bacterial overgrowth, 240–242
laboratory detection of, due to maldigestion, 238–239
Maldigestion, laboratory detection of malassimilation due to, 238
Malignancy, cytomorphologic features of, 340*t*
Malignant lymphoma, 87
Malignant melanoma, *337*
Mammalian leukocytes, 45
Mammals
age-related changes in plasma proteins in, 173
erythropoiesis in, 9
neutropenia in, 71–73, 72*t*
neutrophilia in, 64–71
Mammary carcinoma cells in lymph node, metastatic, *343*
Mare colostrum, antibodies in, 25
Marginal neutrophil pool, 51
Mast cell leukemia, 100
blood of dog, *86*
Mast cells, 55–56
in blood of dog with enteritis, *48*
function of, 55–56
morphology and production of, 55
Mast cell tumors, 341
cell, *337*
of nasal cavity, 357, 358
Mastitis, acute septic, 438–439
Mastocytemia, 56
MCH. *See* Mean corpuscular hemoglobin
MCHC. *See* Mean corpuscular hemoglobin concentration
MCV. *See* Mean corpuscular volume
MDS. *See* Myelodysplastic syndrome
Mean corpuscular hemoglobin, 14, 15
increased, hyperadrenocorticism and diabetes mellitus, with other complications and, 470
Mean corpuscular hemoglobin concentration, 14, 15
Mean corpuscular volume, 14
Mean platelet component concentration, determining, 114
Mean platelet volume, measuring, 113
Mediastinal lymphoma, 87
in cats, 90
in horses, 90
Medical decision limit, 373
Medullary washout, concentration test results and diseases causing, 274
Megabacteria, of birds, *335*, 338
Megaesophagus
complete blood count and, 244
defined, 243
hypoadrenocorticism and, 243–244
hypothyroidism and, 243
laboratory detection of, 243–244
myasthenia gravis and, 243
Megakaryocyte-erythroid progenitor, 109
Megakaryocytes
derivation of, 109
Stage I, II, and III, 109
Megakaryocytic colony-forming units, *8*
Megakaryocytic leukemia, 98, 123
blood of cat, *86*
Megesterol acetate, hyperglycemia and, 192
Melanomas, 342
malignant, *337*
Membrane-bound cytoplasmic granules, types of, 108–109
Membrane filter test, for detecting IgG, 177
MEP. *See* Megakaryocyte-erythroid progenitor
Mesenchymal/connective tissue tumors, 340
Mesothelial cells, *343*
Metabolic acid base imbalance, disorders of, 156
Metabolic acidosis, 163–165
acute renal failure (ethylene glycol toxicosis) and, 431
acute salmonellosis and, 399
feline lower urinary tract disease and, 437
mechanisms and causes of, 163–165
Metabolic alkalosis, 165–167
causes of, 166
rare, 166–167
paradoxical aciduria in HCl loss, 166
right abomasal displacement and, 444
Metamyelocyte, *49*
Metarubricytes, *9*, *10*
description of, 21
dog, *17*
Metastatic hepatic neoplasia, clinical features and laboratory findings on, 227–228
Metastatic mammary carcinoma cells in lymph node, *343*
Metastatic neoplasia, 347
Methemoglobin, 12
Methemoglobinemia, acute intravascular hemolytic anemia and, 390
Methemoglobin reductase pathway, 7
Metritis, 360–361
Micelles, *184*
Microangiopathic anemia, 35
Microbicidal action, neutrophils and, 51
Microcystosis, hepatic failure and, 417
Microcytes, description of, 18
Microcytic, hypochromic anemia, with variable neutrophil and platelet counts, 39
Microcytosis without anemia, 40
Microfilariae, 21
Microplatelets, 113
Microscopic evaluation, of blood smear, 15–16 59
Mineralocorticoid deficiency, 319
Miniature horses, hyperlipidemia of enterocolitis in, 189
Miniature Poodles, PK deficiency in, 5
Miniature Schnauzers
pancratitis in, 237
stomatocytosis with macrocytosis in, 15
Mixed metabolic acidosis and alkalosis, laboratory findings, mechanisms, and causes of, 167
Mixed metabolic and respiratory acidosis, end stage renal disease with uremic pneumonitis and, 463–464
Mixed respiratory and metabolic acid-base disorders, 169
MNP. *See* Marginal neutrophil pool
Modified Lowry (bicinchonic acid) method, for total protein measurement, 175
Modified transudates, 347, 349–350

www.Ebook777.com

Monoclonal gammopathy, 181
Monocyte colony-forming units, *8*
Monocytes, 45
  avian, *46*
  canine, three, *48*
  function, 53
  morphology, 52
  production and kinetics, 53
Monocytic leukemia, 97
  blood of dog, *85*
Monocytopenia, 74
Monocytosis
  corticosteroid or "stress" leukogram and, 236
  description and mechanisms of, 74
  feline infectious panleukopenia and, 406
  neutrophilia and heterophilia of inflammation and, 68
  rodenticide toxicosis and, 448
Morphine, hyperglycemia and, 192
MPC. *See* Mean platelet component concentration
MPV. *See* Mean platelet volume
Mucin, large amorphous masses of, *336*
Mucin clot test, 352–353
Mucus, in urine sediment, 270
Mucus thread, in urinary sediment, *266*
Multicentric lymphoma, 87
  bovine, 91
  equine, 90
  feline, 89
Multicentric lymphoma (case 10)
  laboratory data, *408–409*
  problems and summary, 409
  signalment, presenting problems, 408
Multifocal hepatic necrosis
  biochemical profile, 228*t*
  clinical features, characteristics, and laboratory findings on, 224
Multipotential stem cells, 7
Muscle, 283–289
  diseases with high serum enzyme activity of muscle origin, 286*t*
  serum enzymes of muscle origin, 283–285
Muscle disease
  laboratory findings in, 287–289
    acetylcholine receptor antibodies and myasthenia gravis, 289
    dystrophin, 289
    erythrocyte glutathione peroxidase activity and selenium deficiency, 289
    lactate, 289
    myoglobin, 288
    natriuretic peptides, 288
    potassium, 289
    thiamine deficiency, 289
    troponins, 287–288
Muscle disease, myoglobinuric nephrosis (case 17), 427–429
  laboratory data, *427–428*
  problems, 428–429
  signalment, presenting problems, 427
  summary, 429
Muscular dystrophy, dystrophin and, 289
Mustelid lymphoma, anatomic, functional, and laboratory features, 90
Myasthenia gravis
  acetylcholine receptor antibodies and, 289
  megaesophagus and, 243
Mycobacteria spp., 53
Mycoplasma haemocanis, 21
Mycoplasma haemofelis, *17*, 21
Mycoplasma haemosuis, 21
Myeloblasts, *10*, 48, *49*
Myelocytes, *49*
Myelodysplastic syndrome, 98
  anemia, maturation abnormalities and, 37
Myeloid: nucleated erythroid cells (M:E ratio), estimating, 24
Myeloid-derived macrophage, 100–101
  definition, 100–101
  subgroups, 101
Myeloid stem cell, 7, *8*
Myelomonocytic leukemia, 97
Myelomonocytic leukemia, blood of horse, *85*
Myelophthisic anemia, 38–39
Myelophthisis, thrombocytopenia and, 121
Myeloproliferative disorders, 95–101
  acute myeloid leukemia, 96–98
  chronic myeloid leukemia, 99–100
  description of, 95
  general characteristics, 96
  mast cell leukemia, 100
  myelodysplastic syndrome, 98
  myeloid-derived macrophage and dendritic cell neoplasms, 100–101
  platelet defects and, 120
Myoglobin
  defined, 288
  occult blood test and, 261
Myoglobinuria
  muscle disease, myoglobinuric nephrosis and, 428
  muscle injury and, 288
  occult blood test and, 262
Myoglobinuric nephrosis, 427–429

N
N-acetyl-beta-D-glucosaminidase, urine activity and, 264
NAHLN. *See* National Animal Health Laboratory Network
Nasal lymphoma, in cats, 90
National Animal Health Laboratory Network, 368
National Cancer Institute of Working Formulation, 87
National Veterinary Service, 368
Natriuretic peptides, 183
  muscle function/dysfunction and, 288
Necrotizing pancreatitis, hyperadrenocorticism and diabetes mellitus with, 468–471
Needle smears, 333
NEFAs. *See* Nonesterified fatty acids
Negative acute-phase protein, 179
Negative likelihood ratios, 380
Negative predictive values, 380, 381
Neoplasia
  biochemical profiles of hepatic disorders in animals with, 228*t*

Free ebooks ==> www.Ebook777.com

effusions and, 351
metastatic, 347
pseudohyperparathyroidism and, 300
serum lipase activity and, 233
Neoplasms, 340–342
classification of, 340
epithelial tumors, 340–341
respiratory tract, 359
round cell tumors, 341–342
spindle cell tumors, 341
vaginal, 361
Neoplastic cells, marker expression of, 88
Neoplastic mastocytemia, 56
Nephrogenic diabetes insipidus, concentration test results and, 274
Nephrosis and perirenal hemorrhage (case 22), 440–442
laboratory data, *440–441*
problems, 441–442
signalment, presenting problems, 440
summary, 442
Nephrotic syndrome, 433–435
description of, 280
enhanced platelet function and, 120
secondary hyperlipidemia and, 189
Nerium oleander, 161
Neutropenia
acute salmonellosis and, 398
in mammals, 71–73
causes of, 72*t*
excessive ineffective granulopoiesis, 73
excessive tissue demand for or destruction of neutrophils, 71
margination of circulating neutrophils, 71
reduced production of neutrophils, 71–73
mechanisms of, *73*
Neutrophilia
end stage renal disease with uremic pneumonitis and, 463
feline infectious peritonitis and, 401
hyperadrenocorticism and, 451
hyperadrenocorticism and diabetes mellitus, with other complications and, 470
hyperthyroidism and, 454
in mammals, 64–71
causes of, 65*t*
clinical findings at time of blood collection, 64
corticosteroid-induced, 66–68
inflammation and infection, 68–71
leukogram findings, 64
mechanisms of, 64, *66*
other causes of, 71
species characteristics, 65
other causes of, 71
rodenticide toxicosis and, 448
urea toxicosis and, 459
Neutrophilia with degenerative left shift, pyometra and, 403
Neutrophilic inflammation, *344*
Neutrophilic leukocytosis
acute intravascular hemolytic anemia and, 390
autoimmune hemolytic anemia and thrombocytopenia with, 388
chronic hemorrhagic anemia and, 395
hemorrhage and, 29
in hemorrhagic anemia, 385
right abomasal displacement and, 444
Neutrophilic leukocytosis and lymphopenia, acute intravascular hemolytic anemia and, 390
Neutrophil kinetics, in blood, 51
Neutrophil maturation stages, 45, *49*
Neutrophil metamyelocytes, *10*
Neutrophil myelocyte, *10*
Neutrophil nuclear hypersegmentation, causes of, 60
Neutrophil nuclear hyposegmentation, 60
Neutrophil release, from bone marrow, 51
Neutrophils, 45
Anaplasma phagocytophilum morulae in cytoplasm of, *62*
canine, *46*
from cat with Pelger-Huet anomaly, *46*
cytology, body cavity effusions, 349
equine, *46*
equine segmented, 49
function of, 51–52
immature, differential leukocyte count and, 59
lymphadenitis and, 347
morphology, 45
production of, 50–51
during proestrus, *346*
toxic, 60
toxic change of, 59
Neutrophil specific antigen, 97
New methylene blue, staining of peripheral blood smear with, 15
Nifedipine, hyporesponsive platelets and, 120
Nitric oxide release, 107
Nitrite test, bacteria in urine and, 263
NMB. *See* New methylene blue
Nocardia spp., filamentous beaded bacteria typical of, *335*
Nodular pattern of lymphoma, 87
Non-*Anaplasmataceae* spp. bacteria, in leukocytes, 61
Nonenzymatic protein coagulation factors, synthesis of, 125
Nonesterified fatty acids, 184, 185–186, 187
Noninflammatory, nonneoplastic lesions, 339–340
Nonregenerative anemias, 4, 27–28
infectious agents as cause of, 39
unknown mechanisms of, 38
Nonseptic inflammation, 353
Normoalbuminemia, chronic hemorrhagic anemia and, 396
Normocytic, normochromic, regenerative anemia rodenticide toxicosis and, 448
Normocytic, normochromic anemia, 37
Normocytic, normochromic anemia with neutropenia, 38
Normoglycemia
glucosuria and, 260
urea toxicosis and, 461
Normokalemia, feline infectious panleukopenia and, 407
Normonatremia, 167, 167*t*
Normonatremic dehydration
acute renal failure (ethylene glycol toxicosis) and, 432
feline infectious panleukopenia and, 407
Normoproteinemia, chronic hemorrhagic anemia and, 396
Normouricemia, 278

www.Ebook777.com

nRBCs. *See* Nucleated erythrocytes
NSA. *See* Neutrophil specific antigen
NTproANP, 288
NTproBNP, 288
Nucleated erythrocytes, 21
  description of, 20
  identifying, 59
Nutritional deficiencies, anemia, maturation abnormalities and, 37
Nutritional (or malabsorption secondary) hyperparathyroidism, *296*

O
Obesity, 190
Occult blood test, 261–262
  hemoglobinuria, 261
  measurement of, 262
  physiology, 261
Odds ratio (OR), 380
Oral glucose tolerance test, 195
Oronasal fistulas, 357
Osmolality
  defined, 146
  extracellular, 146–147
  of urine, 255
Osmolal or osmolar gap, 146
Osteolysis, 94
Osteosarcomas, nasal, 357
Ovine erythrocytes, morphology, 18
Oxidants, erythrocytes and, 35

P
Packed-cell volume, 13
PAF. *See* Platelet activating factor
PAI. *See* Plasminogen activator inhibitors
Pancreas, functions of, 315
Pancreatic disease, laboratory tests specific for, 234–235
Pancreatic inflammation and necrosis
  complete blood count, 235–236
  peritoneal fluid evaluation, 236–237
  serum biochemical abnormalities, 236
  supplementary laboratory findings in, 235–237
  urinalysis, 236
Pancreatic lipase immunoreactivity, 234
Pancreatitis
  acute, hepatic lipidosis and, 225
  in cats, diagnostic steps for, 237–238
  clinical spectrum of, 231
  in dogs, diagnostic steps for, 237
  hypocalcemia and, 301
  laboratory detection of, 231–238
    serum enzyme activity measurement in general biochemical profiles, 231–234
    specific tests for pancreatic disease, 234–235
Pancytopenia, estrogen-induced, 392–393
Panhypoproteinemia, enteric disease and, 244
Paradoxical aciduria, 280
  right abomasal displacement and, 444
Paraproteinemia, 181
Paraproteinemia of plasma cell myeloma, hyporesponsive platelets and, 120
Paraquat toxicosis, acute, in dog, 445–446
Parasite ova, in urine, 271
Parasites
  epicellular, 21
  extracellular, 21
  intracellular, 21
Parathormone
  major disorders involving, *296*
  production of and activities promoted by, 295
  quantitation of, 297
Parathormone-related peptide, 295, 297
Parathyroid function, 295
Parentage testing, antigen identification and, 24
Parrots, xanthomas in, 338
Partial pressure of carbon dioxide (PCO_2), 148
Parvovirus enteritis, secondary hyperlipidemia and, 189
Parvovirus infection, in cat, 405–407
PCV. *See* Packed-cell volume
PDGF. *See* Platelet-derived growth factor
PDW. *See* Platelet distribution width
Pelger-Huet anomaly, 60
Penicillium spp., nasal infections and, 357
Pentose phosphate pathway, 7
Peracute purulent inflammatory disease, acute septic mastitis and, 439
Pericardial effusions, in dogs, 351
Peripheral blood smear, 15–21
  staining and examination of, 15–16
  systemic evaluation of, 16
Perirenal hemorrhage, 440–442
Peritoneal fluid evaluation, pancreatic inflammation and necrosis and, 236–237
Peritonitis, serum lipase activity and, 233
Perl's blue staining, bone marrow examination and, 24
Persistent hyperglycemia, 195
Persistent hyperlipidemia, 187
PFK. *See* Phosphofructokinase
PFK deficiency, consequences of, 7
pH, of urine, 258, 263
Phagocytosis, neutrophils and, 51
Phagocytosis by macrophages, senescent erythrocyte removal and, 11, *12*
Phenobarbital administration, primary hypothyroidism and, 467
Phosphatidylserine, 125
Phosphofructokinase, 5
Phospholipids, 184
Phosphorus
  fractional clearance of, 279
  function of, 299
  laboratory abnormalities in, 299–304
  major disorders involving, *296*
Phylloerythrin, 223
Physiologic thrombocytosis, 122
Pigs, suckling, reticulocytosis in, 22
Pituitary diabetes insipidus, concentration test results and, 274
PK deficiency, characteristics of, 5
Plasma, color of, 13
Plasma carbohydrates, 190–200
  glucose, 191–196

Free ebooks ==> www.Ebook777.com

ketones, 197, 199
lactate, 199–200
Plasma cell myeloma, 93, 94
bone marrow, dog, *84*
Plasma cell tumors, 93–95
definition and occurrence, 93–94
diagnostic criteria, 94
laboratory features, 94–95
Plasma cortisol measurement, adrenal cortex evaluation and, 319
Plasmacytomas, 93, 94, *337*
Plasma lipids, 183–190
hyperlipidemia, 187–190
measurement and clinical significance of, 186–187
types and origins, 183–186
Plasma lipoproteins, characteristics of, 185
Plasma protein concentration, hemorrhage and, 29
Plasma proteins, 173–175
age-related changes in, 173
in fresh serum, 173
nutritive function of, 173
Plasmin, plasminogen activation to, 129
Plasminogen, 129
activators of, intrinsic and extrinsic, 131
Plasminogen activator inhibitors, 131
Plasminogen activator inhibitor type 1, 108
Plasmodium spp., 21
Platelet activating factor, 111
Platelet aggregation, 16, 114
Platelet aggregometers, 114
Platelet counts
automated, 111–112
hemorrhage and, 29
interpreting, 112
manual count with hemocytometer, 112
Platelet-derived growth factor, 111
Platelet distribution width, determining, 113
Platelet estimation, determining, 112–113
Platelet Function Analyzer-100, 114
Platelet function assays, 114
Platelet glycoprotein Ib-IX-V, 110
Platelet microparticles, 113
Platelet morphology, *17*
Platelet phospholipid, 125
Platelet plugging, clot retraction evaluation scale, *116*
Platelets, 107, 108–123
circulating lifespan for, 109
disorders of, 117–123
acquired qualitative functional disorders, 120–121
causes of thrombocytosis, 122–123
extrinsic platelet dysfunction, 117–118
functional impairment of platelets, 117
intrinsic platelet dysfunction, 118–119
mechanisms of thrombocytopenia, 121–122
thrombocytosis, 117
function of, 110–111
adhesion, 110
aggregation, 110
granule release, 110–111
shape changes, 110
laboratory evaluation of, 111–117
antiplatelet antibody, 114
bleeding time, 115
clot retraction, 115
flow cytometry assays, 116–117
mean platelet component concentration, 114
mean platelet volume, 113
platelet count, 111–112
platelet distribution width, 113
platelet evaluation from stained blood smears, 112–113
platelet function assays, 114–115
reticulated platelets, 113
VWF assays, 116
mammalian, description of, 108
morphology, 108–109
production of, 109–110
Platelet surface-associated immunoglobulin, direct determination of, by fluorescence labeling and flow cytometry, 114
PLE. *See* Protein-losing enteropathy
Pleural effusion, hyperthyroidism and, 455
PLI. *See* Pancreatic lipase immunoreactivity
Pluripotential stem cells, 7, *8*
Poikilocytes, types of, 19–20
Polychromasia
description of, 18
dog, *17*
Polychromatophilic erythrocytes, 18, 19
with Howell-Jolly body, *10*
Polyclonal gammopathies, characteristics of, 181
Polycythemia, 40–41
absolute, 41
acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
acute renal failure (ethylene glycol toxicosis) and, 431
acute salmonellosis and, 398
hyperthyroidism and, 454
spurious or relative, 41
urea toxicosis and, 459
Polycythemia vera, 99–100
Polyomavirus, heteropenia in birds and, 72
Polyuria, concentration test results and diseases causing, 274
Poodles
congenital macrocytosis in, 14
methemoglobin reductase deficiency in, 7
Porcine eosinophil, *47*
Porcine erythrocytes, morphology of, 16
Porphyrins, heme biosynthesis and, 3
Portal blood flow, alteration of, 212
Portosystemic shunts
congenital, 227
microcytosis with mild anemia and, 40
Portosystemic venous shunt with hepatic atrophy (case 13), 415–417
laboratory data, *415–416*
problems, 416–417
signalment, presenting problems, radiographic findings, 415
summary, 417
Positive acute-phase protein, 179

www.Ebook777.com

Positive likelihood ratios, 380
Positive predictive value, 380
Post-analytical test errors, 366
Postprandial hyperlipemia, secondary hyperlipidemia and, 188
Postprandial hyperlipidemia, 187
Postrenal azotemia, 276
Post-test odds, 380
Potassium
  fractional clearance of, 279
  muscle and, 289
Potassium depletion, urea toxicosis and, 460
Poultry, erythroleukemia in, 98
PRCA. *See* Pure red cell aplasia
Pre-analytical test errors, 366
Precision, test validity and, 366
Precursor cells, 7
Predictive values
  negative, 380, 381
  positive, 380
Prerenal azotemia, 275–276
  feline infectious panleukopenia and, 406
Prerenal proteinuria, 259
Pre-test odds, 380
Primary absolute polycythemia, 41
Primary granules, 45
Primary hepatic neoplasia, clinical features and laboratory findings on, 227–228
Primary hypercholesterolemia, 188
Primary hyperlipidemia, 187, 188*t*
Primary hyperparathyroidism, *296*
Primary hypoparathyroidism, *296*
Proconvertin, 124
Proestrus
  cytologic characteristics of, 360
  noncornified epithelial cells and neutrophils during, *346*
Proetinuria, diabetes mellitus and, 317
Progenitor cells, 7, 9
Promonocytes, 52
Promyelocytes (progranulocytes), *10*, 48, *49*
Promyelocytic leukemia, 97
Prorubricyte, *9*
Prostacyclin release, 107
Protein
  in cerebrospinal fluid, 355
  measurement of, 175–177
    acute phase protein methods, 177
    albumin, 176
    albumin/globulin (A/G) ratio, 177
    fibrinogen, 177
    globulin, 176–177
    total protein, 175–176
  in urine, 257
Protein abnormalities (dysproteinemias), 177–182
  hyperprotenemia, 177–181
  hypoproteinemia, 181–182
Protein-bound calcium, 297
Protein C, 107, 128–129, 137–138
Protein-losing enteropathy, 244–245
  fecal alpha$_1$-proteinase inhibitor immunoassay and, 244
  hypocalcemia and, 245
  hypoproteinemia and, 244
  lymphangiectasia and, 242
  quantifying radiolabeled protein loss into intestinal tract, 245
Protein S, 107
Protein synthesis, hepatic function evaluation and, 212
Proteinuria
  acute renal failure (ethylene glycol toxicosis) and, 431
  end stage renal disease with uremic pneumonitis and, 464
  hepatic failure and, 417
  interpretation positive test result for, 258
  measurement of, 258
Proteus, equine hyperammonemia and, 245
Prothrombin, 124
Prothrombinase complex, 128
Prothrombin time, 135–136
  clotting factor activity and, 224
Prototheca spp., 339
  in urine, 271
Protozoa, 339
Prussian blue staining, bone marrow examination and, 24
PSAIg. *See* Platelet surface-associated immunoglobulin
Pseudocasts, 270
Pseudohyperparathyroidism, neoplasia-associated, 300
Pseudomonas spp., equine hyperammonemia and, 245
Pseudoneutropenia, 71
Pseudo-Pelger-Huet anomaly, 60
Pseudothrombocytosis, artifacts of lipemia, hemolysis, and improper sample handling and, 473
PSS. *See* Portosystemic shunts
PT. *See* Prothrombin time
PTH. *See* Parathormone
PTHrp. *See* Parathormone-related peptide
P2Y12, 119
Pugs, PK deficiency in, 5
Pulmonary carcinoma, *346*
Pulmonary hemorrhage and edema (acute Paraquat toxicosis) (case study), 445–446
  laboratory data, *445*
  problems, summary, 446
  signalment, medical history, presenting problems, 445
Pulmonary lesions, fungi and, 359
Pulse oximetry, 149–150
Punctate reticulocytes, in cats, 22
Pure megakaryocytic hypoplasia, 121
Pure red cell aplasia, 38
Purulent exudates, 333
Purulent inflammation
  causes of, 359
  with nasal exudates and masses, 357
Purulent peritonitis, feline infectious peritonitis and, 401
Pyogranulomatous inflammation, ruptured keratin-producing cyst or tumor and, *336*
Pyometra (case 8), 402–404
  laboratory data, *402–403*
  problems, 403–404
  signalment, history, presenting problems, 402
  summary, 404
Pyothorax, degenerate neutrophils and mixed bacteria from, *344*

Free ebooks ==> www.Ebook777.com

Pyridoxine deficiency, iron deficiency anemia and, 40
Pyruvate kinase. *See* Adenosine triphosphate
Pyuria, detecting, 263

Q
Quality assurance, in laboratories, 368
Quality control
  test results and, 367–369
    commercial and institutional laboratories, 367–368
    in-clinic testing, 368–369
Quantitative buffy coat analysis, platelet counts and, 111–112

R
Racing Greyhounds, acid-base balance and electrolytes in, 155
Radioimmunoassays, 177
Rambouillet sheep, inherited vitamin-K-dependent multifactor coagulopathies in, 129
Rank-percentile method, for reference interval calculations, 371
Rapid-reacting acute-phase proteins, 179
Rapoport-Luebering pathway, 7
Ratites, urine transparency in, 255
RBC agglutination, 16
RBC counts, factors affecting, 14
RBC rouleaux formation, 16
Reactive thrombocytosis, 123
REAL. *See* Revised European-American Classification of Lymphoid Neoplasms
Receiver operator characteristic (ROC) curve, 379, *379*
Recombinant human TSH, 310
Rectal scrapings, pathogenic organisms found in cytologic preparations of, 246
Red blood cell antigens/antibodies, general concepts related to, 24–25
Red blood cell indices, 14–15
  mean corpuscular hemoglobin, 15
  mean corpuscular hemoglobin concentration, 15
  mean corpuscular volume, 14
  red cell distribution width, 15
Red cell distribution width, 15
Red maple toxicosis, in horse, 389–391
Reduced erythropoiesis
  anemia from
    causes of, 36*t*
    differentiation of, 37–40
    general considerations, 36–37
Reference group
  demographic parameters for, 370
  environmental and physiological conditions for, 370
Reference intervals, 369–373
  calculating, methods for, 370–371
  chemistry, 374–375*t*
  hematology, 372*t*
  hemostasis, 373*t*
  narrow, 371
  reference group and, 370
  specificity and, 369–370
  specimen collection and handling parameters, 370
  transfer of, 371, 373
  wide, 371
Refractometry
  protein measurement with, 175
  urine specific gravity measurement and, 256
Regenerative anemia, 27
Relative lymphocytes, 60
Relative polycythemia, causes of, 41
Renal abnormalities
  laboratory test detection of, 272–280
    definitions, 272–273
    evaluation of renal function, 273–280
Renal abscess and secondary disseminated intravascular coagulation (case 11), 410–412
  laboratory data, *410–411*
  problems, 411–412
  signalment, history, presenting problems, 410
  summary, 412
Renal amyloidosis (nephrotic syndrome) (case 19), 433–435
  laboratory data, *433–434*
  problems, 434–435
  signalment, presenting problems, 433
  summary, 435
Renal azotemia, 276
Renal disease
  concentration test results and, 274
  defined, 272
  in horses, hypercalcemia and, 300
  miscellaneous alterations occurring in, 279–280
  nephrosis and perirenal hemorrhage and, 441
  serum iron and, 4
  urogenital proteinuria and, 259
Renal epithelial cells, in urine sediment examination, 269
Renal failure
  acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
  bovine, 280
  defined, 272
  in dogs, 280
  exocrine pancreatic insufficiency and, 239
  hypocalcemia and, 301
  inadequately concentrated urine and, 256
  multicentric lymphoma and, 409
  muscle disease, myoglobinuric nephrosis and, 429
Renal function evaluation, 273–280
  blood urea nitrogen, 274–277
  GFR measurement, 278–279
  serum creatinine, 277–278
  uric acid, 278
  urine concentration tests, 273–274
  urine electrolyte clearance ratios, 279
Renal functions, measurement of, 273
Renal proteinuria, renal amyloidosis and, 435
Renal secondary hyperparathyroidism, *296*
Renal tubular acidosis, 263
Reovirus infections, heteropenia in birds and, 72
Resistin, obesity and, 190
Respiratory acidosis, 168*t*
  hypoventilatory diseases and circumstances associated with, 168*t*
Respiratory alkalosis, 169, 169*t*

www.Ebook777.com

Respiratory function disorders, 167–169
  arterial PCO_2, 168–169
  arterial PO_2, 167–168
Respiratory system cytology, 356–359
  nasal exudates and masses, 356–358
  tracheo-bronchoalveolar cytology, 358–359
Reticulated platelets, 113
Reticulocyte counts, in hemolytic anemia, 30
Reticulocyte enumeration, means of, 22–23
Reticulocyte percentage, 22
Reticulocyte production index, 23
Reticulocytes, *9*
  defined, 21
  interpretation of parameters for, 23
  quantitation of, 21
  species characteristics of, 21–22
Reticulocytosis, 14, 23
  in cats. *vs.* in dogs, 22
Revised European-American Classification of Lymphoid Neoplasms, 88
Rhinosporidiosis, 357
Rhinosporidium seeberi, 357
  organisms from nasal cavity, *345*
Rhodococcus equi, degenerate neutrophils and, *345*
rhTSH. *See* Recombinant human TSH
Rickettsial diseases, thrombocytopenia and, 121
Rickettsia spp., 53
Right abomasal displacement (case 23), 443–444
  laboratory data, *443–444*
  problems, summary, 444
  signalment, presenting problems, 443
Ristocetin assays, 116
Rodenticide (coumarin) toxicosis (case 25), 447–449
  laboratory data, *447–448*
  problems, 448–449
  signalment, medical history, presenting problems, 447
  summary, 449
Romanowsky stains, 16
Rottweiler dogs, hypereosinophilic syndrome in, 74
Rough-coated Collie, exocrine pancreatic insufficiency in, 238
Rouleaux formations, description of, 18
Round cell tumors, 341–342
  diagnosis of, 341
  histiocytomas, 342
  lymphomas, 342
  mast cell tumors, 341
  melanomas, 342
  of nasal cavity, 357
  plasmacytomas, 342
  transmissible venereal tumors, 342
RPI. *See* Reticulocyte production index
RTA. *See* Renal tubular acidosis
Rubriblasts, *9*, *10*
Rubricyte, *10*
Ruminants, chronic renal disease and hypocalcemia in, 302
Russell viper venom test (Stypven time), 136

S
S. equi lymphadenitis, degenerate neutrophils and chains of bacterial cocci from, *343*
Saliva
  as surrogate for blood in assessing acute-phase proteins, 179–180
  urea excretion via, 275
Salivary gland cells, normal, *343*
Salmonellosis, acute (case 6), 397–399
  laboratory data, *397*
  problems, 398–399
  signalment, presenting problems, 397
  summary, 399
Sample handling, improper, artifacts of lipemia, hemolysis and, 472–474
Sarcocystis spp., 339
SCF, 7
Schistocytes
  defined, 35
  description of, 19
  in dog, *17*
SCID. *See* Severe combined immunodeficiency disease
Scottish Deerhounds, basal serum T4 and fT4 concentrations in, 309
Scottish Terriers
  high mean ALP activity in, 216
  Type 3 VWD in, 118
Scott syndrome, 119
Scurvy, bleeding time and, 115
SDH. *See* Sorbitol dehydrogenase
Sebaceous cells, *336*
Sebaceous gland hyperplasia, 339
Secondary absolute polycythemia, causes of, 41
Secondary disseminated intravascular coagulation, renal abscess and, 410–412
Secondary granules, 45
Secondary hyperlipidemia, causes of, 188–189, 188*t*
Secretory acidosis, 165
Segmenter, granulopoiesis, 49, *49*
Selenium deficiency, erythrocyte glutathione peroxidase activity and, 289
Sensitivity, laboratory tests, 377, 380
Serine protease coagulation factors, synthesis of, 124
Serm amyloid A (SAA), 179
Seromas, 339–340
Sertoli cell tumor, 392–393
Serum, ionic composition of, *153*
Serum chloride, 162–163
  abnormalities, 162
  evaluation of abnormalities, 162–163
  physiologic considerations, 162
Serum cholesterol determination, 186
Serum cobalamin
  malabsorption and, 240
  small intestinal bacterial overgrowth and, 240–241
Serum creatinine, 277–278
  excretion, renal and gastrointestinal, 277
  interpretation of increased serum creatinine concentration, 277–278
  metabolism, 277
Serum electrophoretograms, normal and abnormal, *174*
Serum enzymes of muscle origin, 283–285
  alanine aminotransferase, 284
  aldolase, 285

Free ebooks ==> www.Ebook777.com

aspartate aminotransferase, 284
creatine kinase, 283–284
lactate dehydrogenase, 284–285
Serum ferritin concentration, increases/decreases in, 5
Serum folate, malassimilation disorders and, 241–242
Serum-free T4, measurement of, 308
Serum fructosamine concentrations, of hyperthyroid cats, 314
Serum glutamic pyruvic transaminase, 284
Serum insulin, 194
Serum iron
decreased, conditions with, 4
increased, conditions with, 4
Serum osmolality, 146
Serum potassium, 158–159, 161–162
mechanisms and causes of hyperkalemia, 159, 160*t*, 161
mechanisms and causes of hypokalemia, 160*t*, 161–162
physiologic considerations, 158–159
Serum pyruvic transaminase, 213, 284
Serum sodium, 156–158
evaluation of Na+/K+ ratio, 158
hyponatremia, normonatremia, or hypernatremia and, 156–158
physiologic considerations, 156
Serum sodium concentration and hydration, mechanisms and diseases associated with combinations of, 156–157*t*
Serum triglyceride determination, 186
Serum unconjugated cholic acid, 242
Setaria spp., 21
Severe combined immunodeficiency disease, in Arabian foals, 182
SGPT. *See* Serum glutamic pyruvic transaminase
Shar Pei
microcytic erthrocytes in, 14
microcytosis without anemia in, 40
Sheep, hypomagnesemia in, 304
Shetland Sheepdogs, Type 3 VWD in, 118
Shiba Inu
microcytic erthrocytes in, 14
microcytosis without anemia in, 40
Shift reticulocytes, 22
SI. *See* Serum iron
Sialocele, 339
macrophages from, *336*
Siberian Huskies, benign familial hyperphosphatasemia in, 216
SIBO. *See* Small intestinal bacterial overgrowth
Six Sigma, 367
Small intestinal bacterial overgrowth
increased folate with EPI and, 242
malabsorption and, 240–242
Small intestinal malabsorptive disease, clinical signs in dogs, 240
Smear preparation, 332–333
Sodium, fractional clearance of, 279
Somali cats, PK deficiency in, 5
Sorbitol dehydrogenase
hepatocellular leakage and, 211
hepatocellular leakage enzymes and, 214
increased activity, urea toxicosis and, 460
Specific granules, 45
Specific gravity, of urine, 255, 256
Specificity, in laboratory tests, 378, 380
Specimen collection parameters, reference intervals and, 370
Specimen handling parameters, reference intervals and, 370
Spermatozoa
in urinary sediment, *266*
in voided urine, 271
Spherocytes
description of, 18, 20
immune-mediated anemia, dog, *17*
Spinal lymphoma, in cats, 90
Spindle cell tumors, types of, 341
Spitz thrombopathia, 118
Spleen, circulating platelet mass in, 109
Sporothrix schenckii yeasts, *335*, 338
Spurious polycythemia, 41
Squamous cell carcinomas, 340
nasal, 357
prominent anisocytosis in tumor cells from, *336*
Squamous cells, with adherent *Simonsiella* sp. and other bacteria from oral cavity, *345*
Squamous epithelial cells, in urine sediment, *265*, 269
Squash preparations, 333
Stab method/nonaspiration fine-needle biopsy, 332
Staging, of lymphoma, 88
Stained blood smears, platelet evaluation from, 112–113
Standardbred horses, inherited familial neutropenia in, 73
Starvation
hepatic lipidosis and, 225
hypertriglyceridemia and, 189
ketonuria and, 261
Stem cell factor, 110
granulopoiesis and, 49
Stem cells, 7
Stephanurus dentatus, in urine specimens, 271
Stewart, P. A., SID theory, 154
Stomatocytes, description of, 20
Stromal cell derived factor 1, 110
Strong ion difference (SID) theory
acid-base interpretation based on strong ion difference, 154
limitations of, 154
simplified, 154
usefulness of, 154
Stuart factor (factor X), 124
SUCA. *See* Serum unconjugated cholic acid
Sulfonamide crystals
drug induced in urine, 272
in urine sediment, *268*
Swabs, 332
Sweat, urea excretion via, 275
Synovial fluid analysis, 352–354
appearance, 352
cytologic examination, 353–354
mucin clot test, 352–353
nucleated cell count, 353
protein, 353
viscosity, 352

www.Ebook777.com

T
TAFI. *See* Thrombin activatable fibrinolysis inhibitor
Tamm-Horsfall protein
  casts and, 270
  in urine, 257
TAP. *See* Trypsinogen activation peptide
Target cells, description of, 19–20
TATs. *See* Thrombin-antithrombin complexes
TBW. *See* Total body water
TEG. *See* Thromboelastography
TEG 5000, 138
Test errors, types of, 366
Test results, generating, 365–381
  basic epidemiology, 365, 373–376, 377–381, 377*t*
  quality control, 365, 367–369
  reference intervals, 369–371, 372*t*, 373, 373*t*, 374–375*t*
  test validity, 365–367
Test validity, 365–367
  protocols related to, 366
  sources of error and, 366–367
TFPI. *See* Tissue factor pathway inhibitor
Theileria annulata, 21
Theileria cervi, 21
Theileria mutans, 21
Thiamine deficiency, muscle and, 289
Thiazide diuretics, hyperglycemia and, 192
Thrombin, as positive feedback accelerator of coagulation, 128
Thrombin activatable fibrinolysis inhibitor, 131
Thrombin-antithrombin complexes, 137
Thrombin formation, scheme for, *124*
Thrombin time, 136
Thrombocytes, avian, *46*, 109
Thrombocytopenia
  autoimmune hemolytic anemia and, 386–388
  disseminated intravascular coagulation and, 411
  drug-induced, 122
  immune-mediated, primary and secondary, 122
  mechanisms of, 121–122
  platelet counts and, 112
  vaccine-induced, 122
Thrombocytosis
  causes of, 122–123
  chronic hemorrhagic anemia and, 395
  in hemorrhagic anemia, 385
  nephrosis and perirenal hemorrhage and, 441
  platelet counts and, 112
Thromboelastography, 138
  comparison of TEG tracings from three dogs with varying coagulation status, *138*
  methods, 138
  TEG tracing, *139*
Thrombomodulin expression, 107–108
Thrombopathia, 118
Thrombopoietin, platelet production regulated with, 109–110
Thrombosis, risks for, 117
Thromboxane A_2, 110
Thyrocalcitonin, 297
Thyroid disease, diagnosis of, TSH stimulation and, 311
Thyroid function, 304–315
  anti-T4 and anti-T3 autoantibodies, 310
  anti-thyroglobulin autoantibodies, 309–310
  hyperthyroidism, 313–315
  hypothyroidism, 312–313
  influence of breed on T4 and fT4 concentrations in dogs, 308–309
  measurement of serum-free T4, 308
  measurement of serum T3, 307–308
  measurement of serum T4, 306–307
  overview, 304–312
  possible glucocorticoid suppression of, 466
  secretion and transport of T3 and T4, 304–306
  T4 response to TRH or TSH, 310–312
  TSH concentration measurement, 309
Thyroiditis
  anti-thyroglobulin autoantibodies in dogs with, 309
  in dogs, anti-T4 autoantibodies and, 310
Thyroid Registry, 310
Thyroid stimulating hormone, *305*
Thyrotropin releasing hormone, *305*
Thyrotropin (TSH) concentration, endogenous, measurement of, 309
Thyroxine (T4), *305*
  measurement of serum-free T4 (fT4), 308–309
  measurement of serum T4, 306–307
  secretion and transport of, 304–306
Tissue factor (factor III), 126
Tissue factor pathway inhibitor, 108, 126
Tissue factor synthesis, 108
Tissue imprints, 331
Tissue plasminogen activator, 108
Tissue scrapings, 331
Titration acidosis, 165
TLI. *See* Trypsin-like immunoreactivity
T lymphocytes, 57
TN. *See* True-negatives
Tonicity, 146
Total body water, 145–146
  decreased, 145
  extracellular fluid, 145
  increased, 146
  intracellular fluid, 145
  volume, 145
Total CO_2 content, measurement and components of, 149
Total protein, measuring, 175
Total unconjugated bile acid, 242
Toxic granulation, characteristics of, 60
Toxoplasma gondii, 339
Toxoplasma spp., 53
TP. *See* True-positives
tPA. *See* Tissue plasminogen activator
TPO. *See* Thrombopoietin
Tracheoalveolar washes, *345*
Tracheo-bronchoalveolar cytology, 358–359
Transferrin, 4, 5
Transitional epithelial cells, in urine sediment examination, 269
Transmissible venereal tumors, 342
Transudates, 347, 349
TRH. *See* Thyrotropin releasing hormone
TRH stimulation test, 311–312
  confirming hyperthyroidism and, 315
Triaditis, 238

Free ebooks ==> www.Ebook777.com

Trichloroethylene, thrombocytopenia and, 121
Trichomonas spp., 339
Triglyceride accumulation, hepatic lipidosis and, 225
Triglycerides, 184
Triiodothyronine (T3), *305*
  measurement of serum T4 and, 307–308
  secretion and transport of, 304–306
Triple phosphate crystals, in urine, 272
Troponin C, 287
Troponin I, 287
Troponins
  cardiac disease and isoforms of, 183
  defined, 287
Troponin T, 287
True-negatives, test results, 377
True-positives, test results, 377
Trypanosoma brucei, 21
Trypanosoma congolense, 21
Trypanosoma cruzi, 21
Trypanosoma evansi, 21
Trypanosoma johnbakeri, 21
Trypanosoma theileri, 21
Trypanosoma vivax, 21
Trypanosomes, 21
Trypsin, 235
Trypsin-like immunoreactivity, 235
  diagnosis of exocrine pancreatic insufficiency and, 238
  malassimilation diseases and typical laboratory changes in, 241*t*
Trypsinogen, 235
Trypsinogen activation peptide, 235
TSH. *See* Thyroid stimulating hormone
TSH response to TRH, 312
TSH stimulation test, 310–311
TT. *See* Thrombin time
T suppression test, confirming hyperthyroidism and, 315
TUBA. *See* Total unconjugated bile acid
Tumors, nasal, 357
Turbidometric immunoassays, 177
2 × 2 contingency tables
  epidemiology, 377*t*
  use of, 376

U
Unbound iron-binding capacity (UIBC), 5
Uncompensated disseminated intravascular coagulation, 411, 412
Uncompensated metabolic acidosis, hyperadrenocorticism and diabetes mellitus, with other complications and, 470
Uncompensated mixed metabolic alkalosis/metabolic acidosis, acute pulmonary hemorrhage and edema (acute Paraquat toxicosis) and, 446
Unconjugated bilirubin, 217, 219, 261
Unipotential progenitor cells, 9
Unopette system, 58
Unstained rod-shaped structures, consistent with mycobacteria within macrophages, *334*
Unstained smears, protecting from formulin fumes, 333
Upper limit of detection, test validity and, 366
UP/UC ratio, interpreting, 259
Urates, in avian urine, 254
Urea toxicosis (case 29), 458–461
  laboratory data, *458–459*
  problems, 459–461
  signalment, medical history, presenting problems, 458
  summary, 461
Uremia
  defined, 273
  hyporesponsive platelets and, 120
Uremic pneumonitis, end stage renal disease with, 462–464
Urethral obstruction, hypocalcemia and, 301
Uric acid, *268*, 278
Uric acid crystals, 272
Urinalysis, 253–272
  chemical characteristics, 257–264
    bilirubin, 261
    canine bladder tumor antigen test, 264
    enzymes, 264
    glucose, 259–260
    hydrogen ion concentration, 263
    interpreting positive test result, 258–259
    ketones, 260–261
    leukocyte esterase activity, 263
    methods of measurement, 257–258
    nitrite, 263
    occult blood test, 261–262
    protein, 257
    urobilinogen, 262–263
    urogenital proteinuria, causes of, 259
  collection methods, 253–254
    catheterized specimens, 253–254
    voided specimens, 253
  malabsorption and, 240
  proper sample handling, 254
  sediment examination, 264, *265–269*, 269–272
    algae, 271–272
    bacteria, 270–271
    casts (cylinduria), 270
    epithelial cells, 264, 269
    erythrocytes, 269
    fat, 270
    fungi, 271
    leukocytes, 269
    mucus, 270
    parasite ova, 271
    principles, 264
    sperm, 271
Urinary calcium excretion, calcium measurement and, 298–299
Urinary protein/creatinine (UPC) ratios, hyperthyroidism and, 313
Urinary sediment
  bacteria and leukocytes in, *266*
  Candida sp. yeasts in, *265*
  Capillaria plica ovum in, *265*
  Dioctophyma renale ova in, *266*
  erythrocytes and leukocytes in, *265*
  fat droplets in, *265*
  fungal hypha in, *266*
  granular cast and spermatozoa in, *266*
  hyaline cast in, *266*
  mucus thread in, *266*
  squamous epithelial cells in, *265*
  transitional epithelial cells in, *265*

www.Ebook777.com

Urinary system, 253–280
  renal abnormalities detected by laboratory tests, 272–280
  urinalysis, 253–272
Urinary tract
  hemorrhage into, urogenital proteinuria and, 259
  inflammation into, urogenital proteinuria and, 259
Urinary tract infection, hyperadrenocorticism and diabetes mellitus with, 468–471
Urinary tract inflammation, estrogen-induced pancytopenia and, 393
Urine
  abnormal volume, causes of, 256*t*
  Bence-Jones protein in, 258
  enzyme activity in, 264
  leukocytes in, 263
  pH of, 258, 263
  physical characteristics of, 254–257
    color, 254
    odor, 255
    solute concentration, 255–257
    transparency, 254–255
    volume, 255, 256*t*
Urine concentration tests, 256, 273–274
  interpreting results of, 274
  rationale for, 273
  types of, 273–274
Urine cortisol/creatinine ratio, with high-dose suppression test, 321
Urine electrolyte clearance ratios, fractional clearances, 279
Urine glucose, 194
Urine protein dipsticks, protein measurement with, 355
Urine protein to urine creatinine, calculating ratio of, 258
Urine sediment
  ammonium biurate in, *267*
  bilirubin with hyaline cast, spermatozoa, and leukocytes in, *267*
  calcium carbonate in, *267*
  calcium oxalate dihydrate in, *268*
  calcium oxalate monohydate in, *267*
  cellular cast with hemoglobin staining, *267*
  cystine crystals in, *268*
  sulfonamide crystals in, *268*
  transitional cell carcinoma in
    wet mount, *269*
    Wright's stain, *269*
  triple phosphate in, *268*
  tyrosine in, *268*
  uric acid in, *268*
  waxy cast in, *267*
Urobilinogen, 262–263
  interpretation of findings, 262–263
  measurement of, 262
  physiology, 262
Urogenital proteinuria, causes of, 259
Uroperitoneum, effusions and, 351

V
Vaccine-induced thrombocytopenia, 122
Vacuolation, cytoplasmic, 60
Vaginal cytology, *346*
  cell collection, 360
  cytologic characteristics of estrus cycle
    anestrus, 360
    diestrus, 360
    estrus, 360
    proestrus, 360
  normal cells, 360
  reproductive diseases, 360–361
    neoplasia, 361
    subinvolution of placental sites, 361
    vaginitis and metritis, 360–361
Vaginal smear, 360
Vaginitis, 360–361
Vascular endothelium, antithrombotic properties of, 107
V-BTA assay, 264
Verapamil, hyporesponsive platelets and, 120
Very-low-density lipoproteins, 185
Veterinary Laboratory Association, 368
Vitamin D, metabolically active, 297
Vitamin D deficiency, hypocalcemia and, 301
Vitamin D metabolism, major disorders involving, *296*
Vitamin-K-dependent clotting factors, liver and production of, 224
Vitamin-K-dependent factors, 124
  acquired deficiency of, 128
  mechanisms and disorders of, 129
VLA. *See* Veterinary Laboratory Association
VLDLs. *See* Very-low-density lipoproteins
Voided urine specimens, 253
von Willebrand factor
  assays, 116
  synthesis, storage, and release of, 108
von Willebrand's disease
  acquired, 118
  case 28, 456–457
    laboratory, *456–457*
    problems, summary, 457
    signalment, presenting problems, 456
  clinical signs of, 117
  forms of, 116
  Type 1, 117–118
  Type 2, 118
  Type 3, 118
VWF. *See* von Willebrand factor

W
Waxy casts, 270
Weibel-Palade bodies, 108
Westgard Rules, 367
West Highland White Terrier, PK deficiency in, 5
White blood cell counts, 58–59
  abnormalities in, 59
  methods of determination, 58–59
  neutrophilia and heterophilia of inflammation/infection and
    in birds, 71
    in cats, 70
    in dogs, 70
    in horses, 70–71
World Health Organization (WHO)

Free ebooks ==> www.Ebook777.com

Classification of Tumours of Haematopoietic and Lymphoid Tissues, 96
lymphoma classification, 88
Wright's stain, 16

X
Xanthomas, 338

Y
Yeasts
Blastomyces dermatitidis, *334*, 338
Candida spp., *346*
Coccidioides immitis, *335*, 338
Cryptococcus neoformans, *334*, 338
CSF with pleocytosis and, *344*
Histoplasma capsulatum, *334*, 338
Malassezia spp., 338
Sporothrix schenckii, *335*
superficial, 338
in urine, 271

Z
Zinc sulfate turbidity test, for detecting IgG, 177

www.Ebook777.com